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Studies on Tuberculous Tracheobronchitis

II. Bronchial Stenosis in Human Pulmonary Tuberculosis

By

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More than two decades have passed since the study on the bronchoscopic appearance of trachea and major bronchi in pulmonary tuberculosis was described in America, during which many a remarkable work was reported. Of late years, other countries have also presented many reports on the bronchoscopic examination in pulmonary tuberculosis. It was in 1948 that our study on the bronchoscopy for tuberculous patients started in Japan, which was followed by the reports that tuberculous tracheobronchitis has a significant importance on the diagnosis and treatment of pulmonary tuberculosis, and particularly that a marked bronchial lesion will exert a bad influence on the collapse therapy[1),2)].

The bronchial lumen produces various degrees of narrowing caused by the extrinsic process of the contraction of neighbouring tissues or the swelling of lymph nodes or collapse therapy other than the intrinsic process due to tuberculous inflammation or cicatrization on the bronchial wall. If these narrowings are in high degree, the influence of bronchial stenosis on parenchymal lesions will be supposed to make the treatment of pulmonary tuberculosis quite difficult. The appearance of antituberculosis agents, such as, streptomycin, PAS, TB_1 and isoniazid have rendered great service to the treatment of tuberculous bronchitis, but a marked tuberculous change of the bronchial wall has the tendency to heal leaving fibroid stenosis after the application of these antituberculosis agents.[3),4)]

With regard to the bronchial stenosis, a number of reports have already been made, but at the present time when fibroid stenosis frequently occurs after tuberculosis therapy or mechanical stenosis after collapse therapy, the author considers it still more important to clarify the relationship of the stenosis with pulmonary tuberculosis.

Concerning the patients in whom stenosis was found by bronchoscopic examination, its incidence and location, the status of tributary parenchymal lesion, and the course of disease according to the type of stenosis and the kind of collapse therapy were studied. The number of examinees was

1,419, on whom bronchoscopic examination was given 2,600 times from August 1948 up to March 1953 at this sanatorium. The incidence and location of the stenosis were examined on both the in- and out-patients, while other items were examined on the in-patients only. The course of these patients was followed up to October 1953, and in the case of discharge by that time, the course during the hospitalization was recorded. The period of observation was five months at the shortest and five years and two months at the longest. A longer period of observation will be required for a conclusion of the prognosis of tuberculosis, but the results of this study will be of some assistance for the present method of treatment of pulmonary tuberculosis having bronchial stenosis.

Statistical Study

In this report, the bronchial lumen in size less than half the lumen of the normal tree of that level was taken as the stenosis with the following classification:

1. Inflammatory stenosis
   a. non-ulcerative inflammatory stenosis
   b. ulcerative stenosis
2. Fibroid stenosis
3. Mechanical stenosis (extrinsic stenosis).

Bronchoscopic examination was given chiefly to the patients suspected to have tuberculous bronchitis clinically or roentgenologically, and also to those who were under the routine examination before and after operative treatment of pulmonary tuberculosis.

Incidence of bronchial stenosis: Out of 1,419 subjects of bronchoscopic examination (2,600 times of examination), 194 (13.7%) were found to have stenosis, that is, 122 (11.6%) of 1,052 males, and 72 (19.6%) of 367 females, higher in the latter. The distribution of stenosis by age is shown in Table I, where 20 to 29 age group seems to be larger in the number of stenosis, which will be due to the larger number of examinees in this group. The oldest age was fifty-five years while the youngest twelve years. Table II indicates the frequency of the stenosis by type. 18 out of 194 patiets had stenosis at two sites while 2 at three sites, so the total number of stenosis was counted as 216. Tuberculous or post-tuberculous stenosis was found in 67 per cent of all, and mechanical stenosis in 33 per cent.

Location of bronchial stenosis (Table III): Stenosis was found more frequently on the right than on the left: most frequently in the right upper lobe bronchus, 38 per cent, next in the left upper lobe bronchus, 25 per cent, and then in the left main bronchus, 12 per cent. Outside of this

TABLE I

Age Distribution of 194 Patients with Bronchial Stenosis

Sex	10 to 19	20 to 29	30 to 39	40 to 49	50 to 59	Total
Male	5	74	31	10	2	122
Female	8	44	16	4	0	72
Total	13	118	47	14	2	194

TABLE II

Frequency of Stenosis by Type

Tuberculous and post-tuberculous	67 per cent
Non-ulcerative inflammatory stenosis	79
Ulcerative stenosis	31
Fibroid stenosis	34
Non-tuberculous	33 per cent
Mechanical stenosis	72
Total	216

TABLE III

Location of Stenosis

Location	Right	Left
Main bronchus	12	26
Upper lobe bronchus	83	52
Intermediate bronchus	3	—
Middle lobe bronchus	5	—
Lower lobe bronchus	9	17
Segmental bronchus	7	2
Total cases	119	97

table, there were 4 cases who had stenosis in trachea.

Parenchymal lesion: X-ray findings of the lung of 152 in-patients at the time when endoscopic diagnosis of bronchial stenosis was made disclosed as follows: 4 minimal cases, 42 moderately advanced cases, 21 far-advanced cases, 83 cases under pneumothorax and operative procedures. Of the remaining 2 cases, one had normal lung roentgenologically with ulcerative stenosis, and the other had mechanical stenosis in the middle lobe bronchus after the right apical and dorsal segmental resection. There was a case who received thoracoplasty on the contralateral side of the lung with stenosis and numbered among thoracoplasty group. In several cases where different kinds of collapse procedure were given contralaterally at the same time, counting was made on the ipsilateral collapse

procedure. The relation between each type of stenosis and the above-mentioned X-ray findings will be seen in Table IV. In case the stenosis exists at two or three sites, the stenosis of the strongest degree was taken for counting.

TABLE IV

Relation between the Type of Stenosis and X-Ray Findings

Type of stenosis	Number of cases	Minimal	Moderately advanced	Far advanced	Pneumothorax treatment	Postthoracoplasty	Post-plombage	Others
Non-ulcerative	59	3	22	9	12	10	3	0
Ulcerative	23	0	5	5	6	2	4	1
Fibroid	22	0	10	6	3	3	0	0
Mechanical	48	1	5	1	9	25	6	1
Total	152	4	42	21	30	40	13	2

TABLE V

Incidence of Atelectasis, Tension Cavity, and Bronchiectasis in Tributary Area of Lung Related to the Type of Bronchial Stenosis

Figures within ( ) indicate X-ray findings before and after the diagnosis of stenosis.

Type of stenosis	Number of cases	Atelectasis	Non-tension cavity	Tension cavity	Bronchiectasis
Non-ulcerative	59	19 (21)	28	6	7
Ulcerative	23	10 (14)	6	0 (1)	3 (4)
Fibroid	22	11 (17)	8	4 (6)	6
Mechanical	48	9 (13)	8	0	12
Total	152	49 (65)	50	10 (13)	28 (29)
%		32 (43)		7 (9)	18 (19)

The frequency of atelectasis, tension cavity and bronchiectasis in the area of the lung distal to the bronchus with stenosis is described in Table V. X-ray findings at the time of the discovery of stenosis revealed that among 152 cases 49 (32%) had atelectasis, and adding 9 cases found at the X-ray examination done comparatively close to that time and also 7 cases found during the course of observation, there waere 65 cases (43%) in all, most frequently in fibroid stenosis type and then in ulcerative stenosis type. Cavity was discovered among 60 cases of which tension cavity existed in 10 cases. The tension cavity was found among the cases of tuberculous

and post-tuberculous stenosis but not in the cases of mechanical stenosis. Bronchiectasis was examined on the only 25 cases who were subject to bronchography, and the remaining cases were examined by their tomogram which showed marked bronchiectasis. It will be noted that the bronchiectasis was frequently found not only in the fibroid type but also in the mechanical type of stenosis.

The Course of Patients Related to the Type of Bronchial Stenosis

The course of 152 patients with reference to the bronchial stenosis type is shown in Table VI. " Satisfactory course " indicates the roentgenological improvement of pulmonary tuberculosis or the successful result of either pneumothorax or operative procedures with absence or slight discharge (occasionally positive by culture test) of tubercle bacilli in sputa. " Unsatisfactory course " represents the aggravation of pulmonary tuberculosis, the unsuccessful results of pneumothorax or operative procedures, or the complication mostly with the presence of tubercle bacilli in sputa. " No change " means the continuation of either satisfactory or unsatisfactory states.

TABLE VI

Course of Patients in Relation to the Type of Bronchial Stenosis

Course of patients 152 cases		Non-ulcerative stenosis 60 cases	Ulcerative stenosis 23 cases	Fibroid stenosis 22 cases	Mechanical stenosis 47 cases
Satisfactory		39 } 41	2 } 2	11 } 12	27 } 35
No change	Satisf.-Satisf.	2	0	1	8
	Unsatisf.-Unsatisf.	8	5	6	6
Unsatisfactory		5 } 19	13 } 21	3 } 10	5 } 12
Dead		6	3	1	1

The most unsatisfactory course was observed in the ulcerative stenosis type, and then in the fibroid stenosis type. The mechanical stenosis type gave comparatively favourable results. 15 of 37 cases of unsatisfactory course and death had the spread of disease in the lung, and others intensive atelectasis, empyema, pleural effusion, etc. Each case of both non-ulcerative and ulcerative types died of suffocation caused by haemorrhage. There was another death from dyspnea following thoracoplasty, which was found due to the contralateral fibroid stenosis. Except one case, the

primary lesions of the lung were always discovered ipsilaterally with the stenosis side. No case was found in whom contralateral pulmonary lesions became worse independently of the ipsilateral lesions or collapse procedures exerting a bad influence on the course of disease.

Collapse Therapy and Pulmonary Resection

Table VII discloses the follow-up results of the course of patients subjected to collapse therapy (pneumothorax 31, thoracoplasty 77, extrapleural leucite plombage 16, pneumoperitoneum 6 including 1 combined with phrenic nerve paralysis) and pulmonary resection (pneumonectomy 3, lobectomy 7, segmental resection 3), together with 30 cases who received neither collapse therapy nor pulmonary resection. The number of patients examined was 152, but counting repeatedly those cases in whom procedures were changed in the course of observation, the number of instances totaled 173. The examinees were divided into two groups, the one which had already been subjected to operative procedure or was under pneumothorax treatment at the time stenosis was discovered, and the other which received these procedures after that time. Non-ulcerative inflammatory, ulcerative and fibroid types of stenosis were observed collectively, but mechanical type separately as shown within parenthesis of the table. Roentgenologically far-advanced cases were found more frequently in the group which had neither collapse therapy nor pulmonary resection than the group which received these procedures. Among other groups, any marked difference was not observed.

The results of treatment was not satisfactory in the pneumothorax group whereas relatively satisfactory in the thoracoplasty group. However, in this group also, bronchiectasis was found in high degree, and there were many instances of post-operative spread of disease or of persistent discharge of tubercle bacilli.

Out of 21 cases whose course of disease became unsatisfactory because of pneumothorax, or whose course was continuously unsatisfactory, 17 were improved after a change of the procedure (Table VIII). In addition to these cases, there were several in whom the course turned satisfactory following the reduction in the amount of air for pneumothorax. When the tables VII and VIII are reviewed, it will be noted that pneumothorax exerts an unsatisfactory effect on the bronchial stenosis. As will be seen in Table IX, 19 cases of unsatisfactory course and death due to pneumothorax had the findings such as extensive atelectasis, pleural fluid accompanying the atelectasis, empyema, cavity persistence, haemothorax, and spread of disease.

Result of extrapleural leucite plombage procedure was less unsatis-

TABLE VII

Results of Collapse Therapy and Pulmonary Resection in Pulmonary Tuberculosis with Bronchil Stenosis

Figures within ( ) indicate the cases of mechanical stenosis.

Course of patient		Without collapse therapy nor resection	Pneumothorax		Thoracoplasty		Plombage before stenosis was diagnosed	Pneumo-peritoneum after stenosis was diagnosed	Pulmonary resection	
			When stenosis was diagnosed	After stenosis was diagnosed	Before stenosis was diagnosed	After stenosis was diagnosed			Before stenosis was diagnosed	After stenosis was diagnosed
Satisfactory		6 (1) } 7	1 (1) } 2	0	7 (14) } 21	23 (4) } 27	2 (0) } 2	3 (1) } 4	1 (0) } 1	8 (2) } 10
No change	Satisf.-Satisf.	1 (2) } 3	0	0	3 (5) } 8	1 (0) } 1	1 (0) } 1	0	0	0
	Unsatisf.-Unsatisf.	10 (1) } 11	6 (4) } 10	0	3 (3) } 6	2 (1) } 3	2 (3) } 5	2 (0) } 2	0	0
Unsatisfactory		5 (0) } 5	12 (5) } 17	0	2 (2) } 4	5 (1) } 6	4 (2) } 6	0	0	1 (0) } 0
Dead		4 (0) } 4	1 (0) } 1	1 (0) } 1	1 (0) } 1	0	1 (1) } 2	0	0	1 (0) } 1
Total cases		30	31		77		16	6	13	

TABLE VIII

Prognosis of 21 Cases in Whom Pneumothorax did not Give Any Satisfactory Results and Another Treatment was Administered

Change of treatment to:	Improved	Unimproved or worse
Discontinuation of pneumothorax	8	2
Thoracoplasty	3	1
Pulmonary resection	3	1
Pneumoperitoneum	2	
Pneumolysis	1	
Total	17	4

TABLE IX

Primary Cause of Unsatisfactory Prognosis due to Pneumothorax

Atelectasis with fluid	6	(including a case of spread of disease)
Atelectasis with empyema	4	
Atelectasis with cavity	3	(including a case of tension cavity)
Atelectasis with hemothorax	2	
Whole unilateral lung atelectasis	2	
Tension cavity	2	(including a case of spread of disease)
Total	19	

factory than that of pneumothorax. Satisfactory results were usually obtained in the group of both pneumoperitoneum and pulmonary resection. In the thoracoplasty group, mechanical stenosis type was found in many cases (Table IV), which will be the cause of satisfactory results. But, it will also be clear from Table VII that in the group of pneumothorax and plombage the unsatisfactory course was observed even in the case of mechanical stenosis. In the thoracoplasty group, better results were obtained than in other groups even with the cases of non-mechanical type of stenosis. The influence of chemotherapy on the course of tuberculosis is very big, and this should always be taken into consideration. However, almost all the cases had already been administered chemotherapeutic drugs, and the average amount of drugs employed was nearly equal among the different procedures, therefore any large influence will not be considered to exert on the course of patients of those groups.

Discussion

In the case of spontaneous healing particularly after chemotherapy, marked tuberculous inflammatory lesions of major bronchi often heal

forming a cicatrization with stenosis stronger than before. This fact has already been reported by us[4] and Hiltz *et al.*[3] The narrowing of major bronchi will occur also after collapse therapy. In this case, it will occur more strongly on the bronchial wall with tuberculous lesion than on the normal one. Under the present examinations, stenosis occurred for the first time after thoracoplasty in 8 cases, the existing stenosis became more manifest after the procedure in one case, and in two cases stenosis disappeared after discontinuation of pneumothorax.

Warren *et al.* (1938)[5] demonstrated 47 cases of ulcer and 16 cases of stenosis out of 71 cases of tuberculous tracheobronchitis while Salkin *et al.* (1943)[6] 33 cases of inflammatory stenosis and 10 cases of fibroid stenosis among 622 pulmonary patients. Out of 466 patients, Huzly (1953)[7] discovered 10 instances of stenosis among 167 nontuberculous bronchus findings, and 24 cases of ulcer and 51 cases of stenosis among 161 tuberculous findings. Lenoine *et al.* (1953)[8] found 188 cases of ulcer and 212 cases of stenosis from 400 patients with bronchial lesion. In the present study, the incidence of stenosis was 194 out of 1,419 tuberculous patients (13.6 per cent); 79 of non-ulcerative inflammatory stenosis, 31 of ulcerative stenosis, 34 of fibroid stenosis, 72 of mechanical stenosis, 216 in total. Although one case of the increase in the number of stenosis discovered will be ascribed to the enlargement of the area exposed to the examination by use of bronchoscopic telescope, the number of both fibroid and mechanical types was larger as compared with ulcerative type. According to Huzly[7], the stenosis was found most frequently in the right upper lobe bronchus, next in the left main bronchus and then in the left upper lobe bronchus, while in the present study its locality was in such order as the right upper lobe bronchus, left upper lobe bronchus, and left main bronchus.

Atelectasis was demonstrated by Hilz *et al.*[3] in 46 of 100 cases of tracheobronchitis, while 12 per cent of 525 cases with normal bronchi and 36 per cent of 97 cases with tuberculous bronchi by Salkin *et al.*[6] The author recognized atelectasis in 32 per cent of 152 cases by means of X-ray findings at the time bronchial stenosis was found, and in 43 per cent including X-ray findings before and after that time. Atelectasis will certainly occur not only by the bronchial obstruction but by other causes also, which is easily supposed from the fact that the atelectasis is often found even when it can not be detected either by bronchoscopy or bronchogram. In this case, the neuromuscular reflex therapy will be taken into consideration as described by Sturm[9] and Alexander[10] *et al.*, but at any rate the frequency of atelectasis is very high when stenosis exists. Of the cases under this study, atelectasis was observed to occur at the time the inflammatory stenosis appeared in one case, and in 3 cases it occurred after the inflam-

matory stenosis turned into tle fibroid one with a marked narrowing. On the other hand, there were 3 cases in which atelectasis disappeared with the disappearance of stenosis.

The effects of bronchial stenosis upon the formation of bronchiectasis were already described by Haslinger in 1928, by Elösser in 1931 and Cohen and Higgins in 1937. The relationship between an obliterative bronchitis and bronchiectasis needs to be stressed. In 15 autopsy cases having tuberculous stenosis, we have observed giant cavity, extensive atelectasis and high degree of bronchiectasis.[11] Similar findings were obtained in 5 clinical and 4 autopsy cases of pulmonary tuberculosis under complete bronchial obstruction.[12] Salkin *et al.*[6] reported on the 3 cases of obliterative bronchitis which changed into bronchiectasis after a period of one year. Among the cases with stenosis in the present report, there was a case who showed marked bronchiectasis after thoracoplasty.

Concerning the mechanism of the occurrence of tension cavity in the cases with stenosis, stress has been put on the check valve action by the American authors and Houghton[13] *et al.* The author has demonstrated the tension cavity in 13 out of 152 cases having bronchial stenosis, not in the mechanical stenosis type but in the tuberculous stenosis type. There was an interesting case in which a large tension cavity existed in the distal parenchymal area of the bronchus having non-ulcerative inflammatory stenosis, and following administration of 20 g. of streptomycin this cavity completely disappeared roentgenologically, but then 10 months later the cavity in the same size emerged at the same site, which disappeared after thoracoplasty in combination with 40 g. of streptomycin. In 2 cases, the tension cavity disappeared by the discontinuation of artificial pneumothorax.

In regard to the results of collapse therapy, pneumothorax was found to be worst, next plombage, and thoracoplasty was rather better. The fact that the result of thoracoplasty is better than pneumothorax has already been reported by Tuttle and O'Brien *et al.*[14] The unsatisfactory result of pneumothorax will primarily due to the occurrence of atelectasis and the complication with pleural fluid and empyema. Reports on the unsatisfactory prognosis of atelectasis due to pneumothorax have already been made by Houghton[13], Maher-Loughman[15], Farquharon[16] and the authors[17][18]. Tuttle and O'Brien *et al.*[14] have administered pneumothorax on 47 cases having tuberculous bronchial stenosis, with the results of 22 cases (46.7%) of unexpandable lung, 15 cases (31.9%) of tuberculous empyema, and 13 deaths. Although this unsatisfactory prognosis was attributed by them to the post-stenotic suppuration, there is still doubt as to the sequelae of retained pyogenic organisms beyond the obstruction[19],[20].

Anyhow, it will be clear that, when there is stenosis, the retension of secretion, the localized atelectasis, and the spread of disease easily occur following collapse therapy. Even in the thoracoplasy groups with relatively satisfactory results, their unsatisfactory prognosis was generally resulted from the spread of disease, bronchiectasis, persistence of cavity in the atelectasis, or the positive tubercle bacilli in sputa.

It has been believed since Coryllos that the occurrence of bronchial obstruction is indispensable for cavity healing, but through the experience in the endobronchial suction and injection for the treatment of pulmonary tuberculosis, the author wishes to agree with Houghton[13] when he says, " I am not happy about the view that the bronchus should close first and that the cavity should heal by inspissation." There will be various opinions on the obstruction of small bronchi and cavity healing, but it is beyond discussion that the stenosis of major bronchus is inexpedient for the betterment of pulmonary lesion, sometimes even deteriorating it. One of the reasons why the result in the plombage group was worse than that in the thoracoplasty group is considered that the major bronchus is pressed more strongly in pombage than in thoracoplasty disturbing the drainage of the major bronchus as in the case of pneumothorax. The satisfactory results of pneumoperitoneum will partly be ascribed to the bronchus-drainage as well.

Result of pulmonary resection was satisfactory except a case in whom bronchial fistula was formed after operation and also a case who died from the aggravated contra-lateral disease after pneumonectomy. It has been supported by many workers that pulmonary resection is useful for the treatment of pulmonary tuberculosis having bronchial stenosis.[21)-24)] Of course there will be a limit in this treatment so far as indication is concerned.

From the above, it will be clear that the pneumothorax is contra-indicated for bronchial stenosis, aud that in case stenosis occurred during the course of pneumothorax, this must be changed to another treatment. Early diagnosis and early treatment is required for tuberculous tracheobronchitis as well as tuberculosis of other organs in order to prevent the occurrence of marked fibroid stenosis.

Summary

1. Out of 2,600 bronchoscopic examinations performed on 1,419 tuberculous patients, bronchial stenosis was found in 194 cases, or 13.7 per cent. If the cases having more than 1 stenosis by themselves were counted repeatedly, the total number of stenosis will become 216.

2. Stenosis was found most frequently in the right upper lobe

bronchus, next in the left upper lobe bronchus, and then in the left main bronchus.

3. X-ray examination of the tributary area of the lung conducted at the time the stenosis was bronchoscopically diagnosed disclosed atelectasis in 32 per cent, tension cavity 7 per cent and bronchiectasis 18 per cent.

4. The course of patients was worst in ulcerative stenosis, next in fibroid stenosis and then non-ulcerative inflammatory stenosis. The course was comparatively satisfactory in the mechanical stenosis group, but it was unsatisfactory in the groups of both pneumothorax and extrapleural plombage.

5. In regard to the results of collapse therapy, pneumothorax was the worst and plombage was the next. As compared with these, thoracoplasty and pneumoperitoneum had better results. The course of 13 cases subjected to pulmonary resection was satisfactory except two cases.

6. In order to obtain satisfactory results of the treatment of pulmonary tuberculosis, thorough considerations should be taken on the presence of stenosis in major bronchi. Further study is needed to prevent the occurrence of stenosis.

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On the Late Results of 435 Cases of Ordinary Gastric Resection for Cancer, with Special Reference to the Relation between the Results and the Histopathologic Findings

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There have appeared an enormous number of reports on gastric resections for cancer and their late results, including a few reports from our clinic (Suzuki,[1] Watanabe and others[2]); but literature which deals fully with the relation between the late results of operation and the histopathologic findings of resected stomachs is comparatively scanty (MacCarty,[3] Saheki,[4] and Imai and Tanaka[5]). Specially taking this point into consideration, I investigated the late results of 435 cases of ordinary gastric resection for cancer in our clinic, with the findings to be reported in the present paper.

Material

Of 976 patients with gastric cancer admitted to our clinic during 11 years and 8 months after April 1941, 959 were subjected to surgical operation. Of these surgical cases, 687 (71.6 per cent) were those of gastric resection (including total gastrectomy and resection of the cardia), in some cases of which remarkable peritoneal carcinomatosis or metastasis in the liver or lymph gland had been found. Excluding 76 cases of total gastrectomy and resection of the cardia, 611 cases (including cases of sub-total gastrectomy) were regarded as cases of ordinary gastrectomy and made the object of the present investigation. Information as to the late results was sought by correspondence with 532 cases, except 79 cases (12.9 per cent) in which the patients had died before discharge from the hospital; but no information could be obtained with 97 cases (18.2 per cent), in 34 cases (6.4 per cent) of which the patients' whereabouts are unknown, most of these patients being inhabitants of the city of Sendai that suffered war

damage. Thus, there have been obtained 435 cases (81.8 per cent) which have afforded data available for the present investigation. The rate of result-traceable cases seems to be higher than those thus for reported.

Results of Investigation

1. *Late results.* The numbers of survivors of the 435 cases were 311 after 1 year (71.5 per cent), 118 after 3 years (37.9 per cent of the cases that had been operated upon before 3 years or more), and 62 after 5 years (29.2 per cent of the cases that had been operated upon before 5 years or more). The survival rates in successive years after operation are shown in Fig. 1. Table I gives those results reported by several authors in our

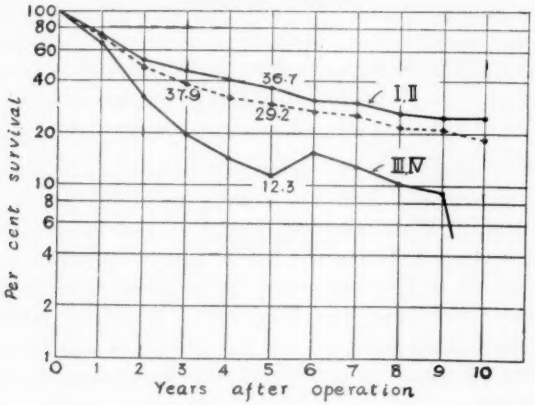

Fig. 1. The survival rates in successive years after gastric resection as calculated for a total of 435 cases (– – – –) and for the macroscopic types of cancer after Borrmann (——). The 5-year survival rates: $\chi^2=11.4$, $P<0.0015$.

TABLE I

Comparison of the Survival Rates in Cases of Gastric Resection reported by Several Authors in our Country

Clinic	Year	Survival rate (%) after	
		3 years	5 years
Miyake Clinic[6]	1933	23.2	17.8
Shiota Clinic[7]	1935	34.8	17.6
Isobe Clinic[8]	1941	29.0	
Akaiwa Clinic[9]	1942	26.3	
Goto Clinic[10]	1948	33.8	22.5
Muto Clinic	1954	37.9	29.2

country for the purpose of comparison.

Recently, the late results of gastric resection for gastric cancer have become more favorable with years; for instance, Walters and others[11] in the Mayo Clinic report that the 5-year survival rate of operated cases during the years between 1940 and 1949 was much higher than that during the years between 1907 and 1916. In the present investigation, survivors of over 10 years are as few as 7; but they are all healthy at present, the longest survival period being 12 years and 2 months.

2. *The sex and the late results.* The 435 cases investigated consisted of 288 males (66 per cent) and 147 females (34 per cent). The 3-year survival rate was 40.6 per cent for males and 33.0 per cent for females, and the 5-year rate was 33.6 per cent for males and 21.8 per cent for females. These differences between both sexes (the rates for males being a little higher) were found to be statistically insignificant.

3. *The age and the late results.* To see if the late results were correlated with the age, the cases were classed in 3 groups according to the age: (1) the under-forty, (2) the forties, and (3) the over-fifty group. The distribution of the cases among the groups was as follows:

The under-forty group	55 cases (13 per cent), including 2 patients as young as 12 and 15 years old.
The forties group	122 cases (28 per cent).
The over-fifty group	258 cases (59 per cent), including 7 seventies (the oldest being 78 years).

It will be seen that the over-fifty cases had a majority of the whole cases. The survival rates of the groups were:

	3 years	5 years
under forty	29.3	16.7
Forties	32.6	28.3
Over fifty	42.7	32.8

It will be found that the survival rates of the under-forty group were lower than those of the over-fifty group, which results agree with those reported in the literature.

4. *The length of the period from presentation of incipient symptoms to operation and the late results.* In 164 (38 per cent) of the 435 cases, symptoms had been present for 6 months or less before operation; and in the remaining 271 (62 per cent), for 7 months or more. The survival rates of the groups were:

	3 years	5 years
6 months or less	33.1	22.5
7 months or more	40.9	32.6

It is to be noted that late results were more unfavorable in cases

where operation had been performed within 6 months before presentation of incipient symptoms, which results are in agreement with those reported by Gray,[12] Balfour,[13] and Ligdas[14], but a little different from those of Tomoda[10], who states that generally the late results are independent of the length of the period before operation though they seem slightly more favorable in the group of a period within 6 months.

This discrepancy may possibly be ascribed to differences in the speed of cancerous growth and in the degree of malignancy according to cases, and in the time of development of symptoms according to the part where a cancerous growth occurs, for example, when a cancer develops in the middle portion of the lesser curvature, symptoms will be revealed first after the growth has reached an appreciable extent.

5. *Macroscopic findings of cancer and the later results.*

i) Size of cancers. Cancers were classified according to the magnitude of the product, the maximum width by the maximum length of a cancer, as follows (the product being conveniently taken to be the area of the cancer): (1) small cancers, which were 19 cm^2 or less; (2) medium cancers, 20 to 39 cm^2; and (3) large cancers, 40 cm^2 or more. The distribution of the cases among these groups and the survival rates of each group were as follows:

Size of cancer	Number of cases	(%)	Survival rate (%) after 3 years	5 years
Small	126	(29)	46.2	35.9
Medium	170	(39)	33.9	21.5
Large	139	(32)	35.3	31.9

It will be seen that there were no great differences in the survival rate among the three groups, though the rates in the group of small cancers were slightly higher.

Balfour[13] found that the five-year survival rate in cases of small cancers was 10 per cent higher than that in cases of large cancers with a few exceptions. Tomoda[10] reports that the smaller a cancer was, the more favorable the late results were. According to Steiner[15] and Imai[5], however, the size of cancers does not seem to be correlated with the late results.

ii) Macroscopic type of cancer. The cases were classified in 4 types (I to IV) according to Borrmann;[16] Types I and II were then combined into one group, and Types III and IV into another. The distribution of cases among the types and the survival rates of the groups (Fig. 1) were as follows.

Type	Number of cases	(%)	Survival rate (%) after 3 years	5 years
I	22	(5)	45.0	36.2

II	282	(66)		
III	91	(21)	19.5	12.3
IV	35	(8)		

The survival rates in the cases of Types I and II will be found clearly higher than in those of III and IV.

Konjetzny,[17] Schildler,[18] Steiner[15] and Imai and Uchinoura,[19] support the view that cancers of Borrmann's I and II Types are benign, while those of III and IV Types are malignant. Thus it may be concluded that there is a close correlation between Borrmann's macroscopic types of cacer and the prognosis.

6. *Histological findings of cancer and the late results.* Two to ten blocks were taken from several places covering the central portion of a cancer. Sections were stained with hematoxilin and eosin.

i) Histologic types of cancer. The histologic type of a cancer was determined by Petersen's classification.[20] When a glandular structure was clearly seen in the central portion of a cancer, it was regarded as carcinoma adenomatosum, even if solid epithelial cords or solid clumps of cells were present in actively growing parts of the margin of the carcinoma; when a glandular structure was not found in any section examined, the cancer was regarded as carcinoma solidum. Those cases in which sections of a block were indicative of carcinoma adenomatosum and those of some other block were of carcinoma solidum, those cases in which an area indicative of carcinoma adenomatosum adjoined an area of carcinoma solidum with a definite border line, and a single case in which the stomach beared two carcinomas (adenomatosum and solidum) at different places were classed as "typus mixtus." The distribution of the cases among the types is shown in Table II.

The ralation of the macroscopic types of carcinoma with the histologic types is shown in Table III. It will be seen that the group of Type I (after Borrmann) included cases of carcinoma adenomatosum more than those of carcinoma solidum, the group of Type II contained both cases in an equal number, and the group of Types III and IV included cases of carcinoma solidum more than the other.

The survival rates of the cases of carcinoma adenomatosum and of carcinoma solidum are shown in Fig. 2: The 3-year survival rate was 53.8 per cent for carcinoma adenomatosum and 26.6 per cent for the other; the 5-year rate was 37.5 and 23.1 per cent, respectively. Thus the survival rates in carcinoma adenomatosum were found to be higher, which was also the case with the rates later than 5 years after operation.

If the cases of carcinoma adenomatosum gelatinosum and carcinoma solidum gelatinosum (Table II) are combined, we obtain 84 cases of

TABLE II

Histologic Classification of Cancer Cases

Type	Cases	
	number	%
Carcinoma adenomatosum:	188	43.2
simplex	101	
microcysticum	31	
papilliferum	26	
gelatinosum	30	
Carcinoma solidum:	226	52.0
alveolare	92	
diffusum	81	
gelatinosum	53	
Typus mixtus:	21	4.8
Total	435	100.0

TABLE III

Relation of the Macroscopic Type (after Borrmann) with the Histologic Type of Cancer

Type	I	II	III	IV
Carc. adenom.	15	135	23	11
Carc. solidum	6	135	60	24
Typus M.	1	12	8	0
Total	22	282	91	35

colloid carcinoma (19.3 per cent). The 3-year and 5-year survival rates were as follows:

	3 years	5 years
Colloid carcinoma	32.2	17.1
Non-colloid carcinoma	40.1	28.8

It might be taken that there were large differences in the survival rate at first sight; these differences, however, were found statistically insignificant because of the smallness in number of cases of colloid carcinoma; but the survival rate later than 5 years arfte operation in cases of colloid carcinoma was significantly lower than in non-colloid carcinoma cases.

ii) The depth of cancerous infiltration through the gastric wall. The 435 cases were classified in 3 groups by the depth of cancerous infiltration through the gastric wall: the groups of (1) Sm Type, in which the infiltra-

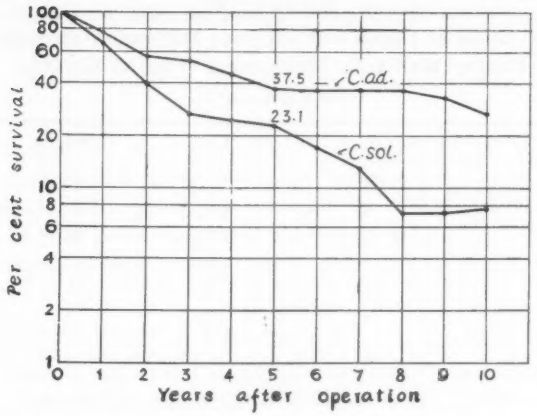

Fig. 2. Comparison of the late results between carcinoma adenomatosum and carcinoma solidum. The 5-year survival rates : $\times^2=4.8$, $P=0.02$

tion was localized in the submucosa, (2) M Type, in which the infiltration extended to the muscular layer, and (3) S Type, in which the infiltration reached the subserous layer. The distribution of the cases among the 3 groups is shown in Table IV, where it will be seen that the cases of Type S had a large majority of the cases.

TABLE IV

Cases as Classified according to Depth of Cancerous Infiltration through Gastric Wall

Type	Cases	
	number	%
Sm	23	5.3
M	73	16.8
S	339	77.9
Total	435	100.0

As for the relation of the depth of infiltration through the gastric wall with macroscopic type of carcinoma, cases of infiltration of the subserous layer (Type S) occurred in any of the Borrmann's macroscopic types, as shown in Table V.

The relation of the depth of cancerous infiltration through the gastric wall with the histologic type of carcinoma is shown in Table VI., where it is to be noted that the groups of Types Sm and M include carcinoma

TABLE V

Relation of Depth of Cancerous Infiltration through Gastric Wall with Macroscopic Type of Cancers

(after Borrmann)

Type	I	II	III	IV
Sm	2	16	1	4
M	6	49	11	5
S	14	217	79	26
Total	22	282	91	35

TABLE VI

Relation of Depth of Cancerous Infiltration through Gastric Wall with Histologic Type of Cancers

Type	Carc. adenom.	Carc. solidum
Sm	20	2
M	35	32
S	133	192
Total	188	226

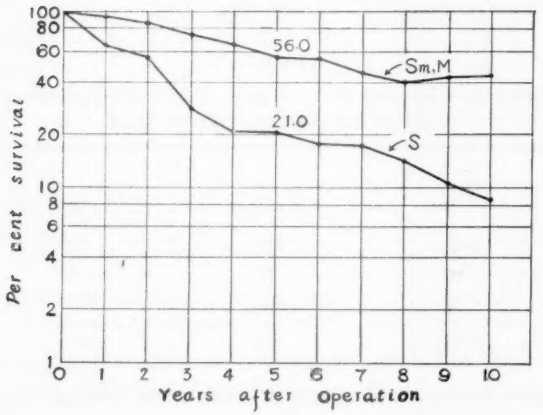

Fig. 3. The relation of the late results with the depth of cancerous infiltration through the gastric wall. The 5-year survival rates: $\chi^2=13.6$, $P<0.0015$.

adenomatosum more in number, while the group of Type S contains carcinoma solidum a little more in number.

A comparison of the survival rates of the groups is given in Fig. 3. The 3-year and 5-year survival rates were as follows:

	3 years	5 years
Sm+M	73.9	56.0
S	27.7	21.0

It will be seen that the survival rates of the group S (of the deepest infiltration) were much higher as was naturally expected.

The 3-year and 5-year survival rates in carcinoma adenomatosum and carcinoma solidum belonging to the group Sm+M (of the infiltration not beyond the muscular layer) were as follows:

	3 years	5 years
Carcinoma adenomatosum	80.0	60.9
Carcinoma solidum	66.7	52.2

It is to be noted that no significant differences existed in the survival rate between both groups. On the other hand, in the group S (of the deepest infiltration) the survival rate of carcinoma solidum was lower than that of carcinoma adenomatosum, as can be seen from the 3-year and 5-year survival rates given below:

	3 years	5 years
Carcinoma adenomatosum	42.9	28.1
Carcinoma solidum	18.4	16.3

As to the relation of the histologic pictures with the late results in gastric cancer, Konjetzny,[17] Schindler[18] and Steiner[15] lay stress on macroscopic findings and hold the view that there is no correlation between the histologic pictures and the prognosis. Petersen[20] found the rates of recurrence after operation of carcinoma adenomatosum, carcinoma solidum and the mixed type to be 88, 68, and 100 per cent, respectively, and states that no correlation existed between the histologic pictures and the prognosis. Contrary to this, MacCarty[3] and Miyake[6] regard carcinoma adenomatosum as benign, and Kazuhara[21] colloid carcinoma to be malignant. The present investigation, in which Petersen's classification was adopted in determining the histologic type of cancer, showed that carcinoma adenomatosum was more benign than carcinoma solidum, differences in the survival rate between both being significant. As for colloid carcinoma, the survival rate was nearly equal to that of non-colloid carcinoma for the first 4 years after operation but was lower thereafter, thus indicating its tendency of malignancy.

As to the depth of cancerous infiltration through the gastric wall, Gray,[12] Miyake[6] and Imai,[5] hold the view that the prognosis are unfavorable for those cases in which cancerous infiltration has extended as far

as the serous membrane; and particularly, Saheki[4] (with gastric cancer) and Majima and others[22-23] (with ulcer-carcinoma) found that the late results were very closely correlated with the depth of cancerous infiltration. My findings agree with theirs in that the late results in cases where solid epithelial cords had attained to the subserous layer (Type S) were unfavorable, and especially in cases of carcinoma solidum of Type S.

Summary and Conclusions

The late results of a total of 435 cases of ordinary gastric resection, including subtotal gastrectomy but excluding total gastrectomy and resection of the cardia, have been investigated from various angles, with the results to be summarized as follows:

1. The general late results of the 435 cases of gastric resection were that the survivors after 3 years were 118 (37.9 per cent) and those after 5 years 62 (29.2 per cent).

2. The relation of the late results with the sex: No significant differences in late results were found between both sexes.

The relation with the age: The survival rate of patients under 40 years old was lower than that of patients over 50 years old.

The relation with the length of the period from presentation of incipient symptoms to operation: The late results of the group undergoing operation within 6 months seem to be more unfavorable than those of the group later than 7 months.

3. The relation with macroscopic findings of cancer: No large differences in the survival rate according to the magnitude of cancerous growths were noticed, but significant differences were found between the groups as classified after Borrmann in that the group including Types III and IV showed a survival rate much lower than the group including I and II.

4. The relation with the histologic type of cancer: The survival rate of cases of carcinoma solidum was lower than that of carcinoma adenomatosum.

5. The relation with the depth of cancerous infiltration through the gastric wall: The survival rate of the cases in which the infiltration attained to the subserous layer was much lower than the rates of the cases in which the infiltration was localized in the submucosa or extended as far as the muscular layer.

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A Case of Diabetes Insipidus Treated with Germanin (Bayer 205)

By

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A 31-years-old miner was admitted complaining of polyuria, thirst, lassitude and impotence since about six years ago. He had suffered from amebic dysentery, dengue fever and malaria at 21 years old. At present: General condition good, acne vulgaris on the face. Urine: Daily amount c. 4,000 c.c., specific gravity 1,005, albumine, sugar and urobilinogen negative, urea 15.78 g. daily. Pyelography normal. The sella turcica diminished in size. Thorn's test abnormal. Aschner's R. positive. Adrenaline sweating threshold 10^{-10} (hyperexitable). Adrenaline test showed the lability of his autonomic nervous system. Blood pressure 152-28. Basal metabolic rate +17%. Wa. R. negative. Mantoux's R. positive. Capillary resistence 10 cmHg. Takata's R. negative. Plasma lipids increased. Serum proteine 7.6 g./dl. (albumine 50%, globuline 50%). Serum chlor 351 mg./dl. Serum urea 9.85 mg./dl.

Administration of pituitary posterior hormone (injection, or implantation of cow's gland) brought only temporary remission and caused side-effects, such as headache and vomiting. But 6 injections of 0.5 g. Germanin cured the side-effects of the pituitary preparation and thirst. A repeat pituitary implantation was made, and 4 further injections of Germanin given subsequently. Urine secretion then decreased and thirst, lassitude, impotence and acne vulgaris regressed. After two months and a half, daily amount of urine was not more than 1,000 c.c. and plasma lipids almost returned to normal prices.

The case of diabetes insipidus treated with Germanin has not yet been reported. It is not clear why this drug showed the curative effect in this patient. However, the auther comfirmed that Germanin could influence the function of the endocrine glands in the animal experiment[1]. This fact seem to give a suggestion to this problem.

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Electromyographical Studies on Graves' Disease

By

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(Received for publication, May 12, 1954)

Recently, the study of EMG has made a considerable advance and its clinical application has become widely applied. The action potential of neuromuscular disorders of exophthalmic goiter has been studied.

Experimental

Method

The action current of motor units from M. biceps brachii using a concentric needle electrode made by Adrian[1] with an oscillograph equipped with a R.C. 4 stage amplifier was recorded.

Usually, normal distribution of spike discharge of human motor units is at random according to Tokizane[2] and do not show any tendency of synchronization or grouping. However, as Denny Brown[3] pointed out already, this distribution is influenced with various conditions, such as instability, fatigue, abnormal sensation, pain and cold weather; and this spike discharge is apt to show the tendency of grouping. In order to avoid the above mentioned phenomenon, the experiment was carried out under the following condition.

1) The patient was told to relax.

2) The test was performed in the early morning when the patient was not tired.

3) 1% novocaine was injected into the skin and fascia to avoid pain. When pain was complained, the needle was reapplied.

4) The patient was confirmed to have no loss of sensation or painful disease.

According to the results which were obtained, even though these conditions were kept in mind, the distribution of spike discharge in a normal person was considered to show a little tendency of grouping, while the action potentials of epileptics without any medical treatment were thought to have scattered spike distribution; therefore the action potential of

epileptics was compared with that of the Graves' disease.

Results

1) In 18 cases of Graves' disease, grouping was observed in EMG according to the strength of tremor. Even in those patients with mild toxic symptoms who didn't show any tremor clinically, the tendency of grouping was observed. The appearance of this kind of grouping was different from that of Parkinson's disease in the following points.

In Graves' disease, grouping appeared only during voluntary contraction, while any abnormal action potential including grouping was not observed at rest. General skeletal muscle also showed grouping during voluntary contraction. However, as already being pointed out by Bishop[4] grouping which was observed in Parkinson's disease was also recorded during involuntary contraction.

When the EMG of antagonistic muscle are recorded simultaneously, the grouping of Graves' disease is synchronous, while that of Parkinson's disease is antagonistic.

In Parkinson's disease, the duration of grouping, the number of the impulses in one group, and the time course of the interval of groups are definite, while they are indefinite in Grave's disease. As the grouping in Graves' disease appears only during voluntary contraction, this character of grouping changes in accordance with the difference of contractions. Besides, in case of Graves' disease with mild toxic symptoms, the formation of grouping is imcomplete and the boundary is often difficult to decide.

2) The relationship of spike discharges of different motor units of which each group consists.

Only 2 or 3 motor units which constitute each group were recorded separately with a concentric needle electrode, and the relationship of spike discharge of different motor units was observed.

The more toxic symptoms were intense, the more spike discharges of different motor units approximated with each other. Finally, a complete grouping was composed. Therefore, in Graves' disease, as like as in Parkinson's disease, the spike discharge of each group was formed by that of different motor units. Could this tendency of approach of spike discharges from different motor units be shown in figures, it would be much more convenient to know the toxic degree of neuromuscular system in Graves' disease. An attempt was made to propose a hypothetic form for this purpose. The statistical method was used, and the following results were obtained.

3) The relationship of the degree of approximation of spike discharge of different motor unit and the severity of toxic conditions.

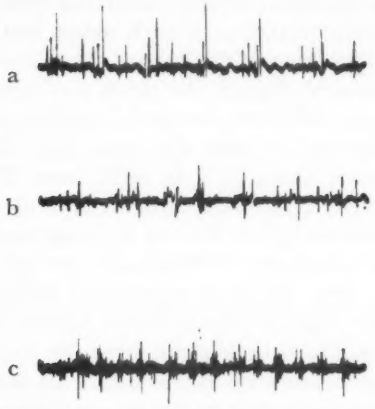

Fig. 1. EMG of M. biceps brachii from a patient of Graves' disease during voluntary contraction. a. Case with slight symptoms (tremor (−)): No tendency of grouping. b. Case with moderate symptoms (Tremor (+)): Tendency of grouping. c. Case with severe symptoms (tremor (++)): remarkable formation of grouping.

In order to measure the approach of spike discharge of different motor unit, appropriate method for analysis was unknown. Therefore, the following statistical method was applied and a hypothetic model is proposed. Fig. 2 shows its model. P unit discharges with a impulse interval, and Q unit discharges with b impulse interval. a & b take any value. P &

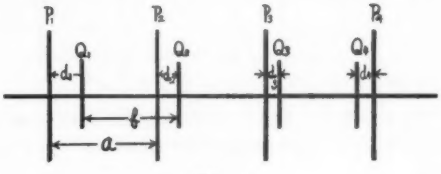

Fig. 2

Q have no correlation between each other. They discharge independently. When spike discharge (P) with a large impulse interval (a) is taken for a standard, d is designated as the distance between the spike discharge Q which is discharging closest to P and the spike discharge P. In this case, it does not matter whether the spike discharges Q locates in front or in the rear of P. Anyhow, the closest discharge to P is taken. In order to read the approximation of (P & Q) regardless its relation to P, the absolute value is taken and designated as d. The arithmetic mean of d is designated as the mean approach ($\bar{d}$). $\bar{d}$ of the EMG of 21 genuine epileptics who show scattered discharge pattern was found to take 1/4 interval mean, i.e.,

a constant b/4. This statistical form is calculated from Weyl's theory, the error area of the interval variation of each motor unit can be easily calculated, because the histogram of the interval shows a resembling Gaussian curve. In case of Graves' disease, the mean $\bar{d}$ of approach d is smaller than b/4; and when the difference between $\bar{d}$ and b/4 is expressed in percentage of b/4, the approach of spike discharges from different motor units is 10–15% in less severe cases, 20% in mild cases, 35% in severe cases.

4) The fluctuation of the impulse interval of single motor unit. Fig. 3 shows the fluctuation by the interval diagram, and Fig. 3a shows less approach of the spike discharge of different motor unit in epileptics; the fluctuation is small. Fig. 3b shows the EMG of Graves' disease with mild toxic symptoms. Fig. 3c shows that of Graves' disease with severe toxic symptoms. Fig. 3b, c both show irregular and rapid fluctuation according to the classification by Hagiwara[5]. Its tendency is more remarkable in the severe cases than in the others. The standard deviation of this irregular and rapid interval variation was calculated. Its relationship with mean impulse interval is shown in Fig. 4. In epileptics, the

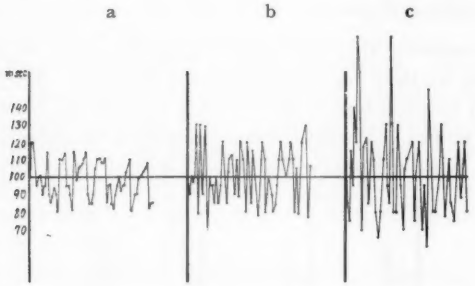

Fig. 3. The time course of the interval variation of a single motor unit. a. Epileptics. b. Graves' disease with slight symptoms. c. A severe case of Graves' disease.

fluctuation increases with the lentgh of mean impulse interval; and it always makes a definite curve. Contrary to epileptics, in Graves' disease, the fluctuation increases more intensely with the length of mean impulse interval than in epileptics. When it is classified in 3 groups according to the strength of grouping, 3 curves are written. This means that an increase of fluctuation of interval is rapid comparing with the slight increase of mean interval, so that clinically a long impulse interval is difficult to obtain. In other words, mean impulse interval becomes shorter. This is coincident with the findings by Sanderson[6]. The increase of fluctuation of the impulse interval is compared with the clinical symptoms.

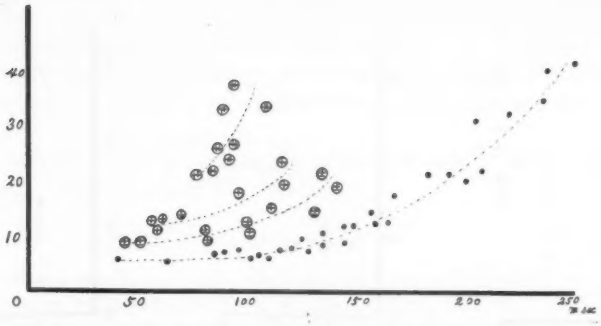

Fig. 4. Relationship between the standard deviation of interval variation of a single motor unit (abscissa) and the mean interval (ordinate). ++ Remarkable grouping in severe case of Graves' disease (percentage of the mean approach to normal: 35%). + Tendency of grouping in moderate degree of Graves' disease (percentage of the mean approach to normal: 20%). ± No tendency of grouping in slight case of Graves' disease (percentage of the mean approach to normal: under 10%). ● Genuine epileptics (percentage of the mean approach to normal: 0%).

The relationship of the standard deviation of the fluctuation of interval of a single motor unit (A) with clinical symptoms (B).

In cases of high basal metabolism, the relationship is shown in Fig. 5. The increase of A and B is parallel. In this figure, standard deviation of fluctuation is influenced by the mean impulse interval. In order to clarify this changes, mark ++, + and ± are used. As being explained previously, both relationship is shown in Fig. 4.

The relationship between the standard deviation and the increase or instability of pulse is shown in Fig. 6 and Fig. 7, respectively. Those which show large fluctuation of impulse interval are accompanied with both increase and instability of pulse.

5) The relationship of the fluctuation of impulse interval of a single motor unit with correlation of two motor units. As being explained in the above paragraph about the increase of interval variation of a single motor unit in case of the tendency of grouping, it is exemplified in the following paragraph.

In Fig. 8, the relationship of approach percentage of spike discharge of two motor units taken by the oscillograph with the value of fluctuation of impulse interval of each unit calculated by standard deviation is shown. Two motor units that were calculated from mean approach were connected by the dotted lines. The more the approach is remarkable, the more the fluctuation of impulse interval of single motor units increases.

Eig. 5 Fig. 6 Fig. 7

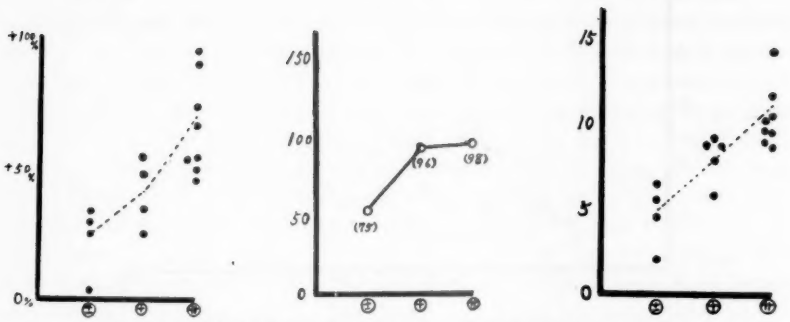

Fig. 5. Relationship between hypermetabolism (abscissa) and the standard deviation of the interval variation dotted line connects the mean value (ordinate).

Fig. 6. Relationship between pulsation per min. (abscissa) and standard deviation of interval variation of a single motor unit (ordinate).

Fig. 7. Relationship between standard deviation of pulse fluctuation (abscissa) and standard deviation of impulse fluctuation (ordinate).

$\bar{\tau}$	S	M (%)
50 54	6.3 7.0	0
96 105	8.3 8.7	0
191 135	20.2 9.0	0
146 138	18.1 16.4	2
100 130	12.7 22.3	20
95 116	19.2 30.0	35

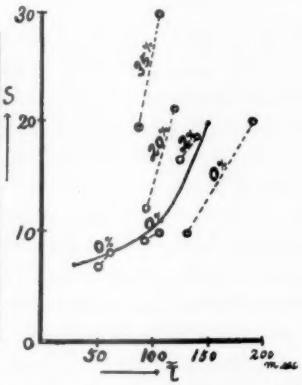

Fig. 8. Showing the standard deviation of interval variation of a single motor unit (S), the percentage of mean approach to normal between two motor units (M) and the mean interval ($\bar{\tau}$).

Summary

The characteristics of the EMG of Graves' disease is that the distribution of spike discharge becomes more irregular than normal during voluntary muscle contraction. This irregularity is due to the following 3 factors.

1. The spike discharges of different motor units are apt to approach.

2. The fluctuation of impulse interval of single motor unit becomes remarkable.

3. Mean impulse interval becomes shorter.

This phenomenon shows a tendency of increase or decrease with toxic symptoms, i.e., high basal metabolism, tachycardia and the instability of the pulse.

It is still unknown how this fluctuation occurs in what part of the motor unit. Further study is required.

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Studies on the Ear-Labyrinth and its Responsiveness to Various Sorts of Medicaments under Phase Contrast Microscope*

By

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We tried to scrutinize the results hitherto obtained in a series of investigations[1)-4)] by means of a phase contrast microscope[5)-6)].

The cells of living organisms are said to contain approximately 90% of water, and if we recall that the ear-labyrinth is especially susceptible to internal and external influences, observation of the tissues in living condition is most desirable.

To begin with, we have tried to examine fresh vital labyrinth tissues under a phase contrast microscope, without killing them by fixation, staining and such manipulations.

In literature, we find that Katsuki and Covell[7)] studied the organ of Corti of guinea-pigs and de Vries[8)] the tectorial membrane of oxen in a similar manner.

Experimental Results and Discussion

As materials of our study, ear-labyrinths were extracted through the opened bullae at room temperature from about fifteen each of rabbits, guinea-pigs and bufones vulgares japonicae. They were immersed in Ringer's solution or cerebrospinal fluid and examined under a phase contrast microscope.

Our experiments were carried out in three steps.

I. At first the histological view of the unmanipulated ear-labyrinths was examined (Figs. 1-9).

Fig. 1: the general view of a Corti's organ.

Fig. 2: External hair cells and the pillar cells under higher magnification.

Fig. 3: External hair cells seen in three rows, containing gourd-

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shaped Deiter's cells among them.

Fig. 4: Internal hair cells, which are flask-shaped in contradistinction to the cylindrical external hair cells.

Fig. 5: Hensen's supporting cells.

Fig. 6: Claudius' cells.

Fig. 7: the stria vascularis, through which blood vessels meander.

Figs. 8 and 9: Otoliths of a guinea-pig and a rabbit respectively.

We see vestibular membranes, macula cells and subcupular fibrils in those figures.

II. The so-called post-mortem changes

In the second step, we traced the course of the so-called post-mortem degeneration of labyrinth tissues.

Fig. 10 shows disintegration of the external hair cells. Swelling appeared in one hour after extirpation of the organ. The Brownian movement of the cell granules, which is indicative of the fluidity of the plasm, was conspicuous up to the third hour, but in most cases, the movement came to a standstill in six hours.

Fig. 11 shows a state when the movement of granules had ceased after five or six hours from the extirpation. The granular structure and the nuclear membranes are distinctly defined. In the left upper portion is seen a probable reticular membrane.

Fig. 12 shows Hensen's cells. Many fat globules are visible. In some cases, the cell boundaries become blurred in about ten hours, as shown in Fig. 13.

In Figs. 14 and 15. A bubble, presumably a hyaline globule, is just coming into view.

Figs. 16 and 17 show such a globule as the one in the former figures breaking out in spherical shape. No nuclei are observable therein.

Fig. 18 shows that the plasmatic membrane has exfoliated. Gaps are found in the membrane.

III. a. Reaction of the membranous tissues against several chemicals

The third step in our experiments was directed to observing the nature of the reactions which the cells in the ear-labyrinth exhibit against the chemicals directly acting upon them.

The tested chemicals and medicaments included 0.1% adrenalin solution, 1–0.1 acetylcholine, 1–0.1% pilocarpine, 5–0.1% acetic acid, 5–1% sodium carbonate, 10% formalin, 5% formol nitrate, Wittmaack's fixer, etc.

Fig. 19: Corti's organ prior to the application of adrenalin.

Fig. 20: Ditto after the application, showing conspicuous contraction of the internal and external sensory cells. Strong contraction occurs upon application of sodium carbonate also, sometimes leading to stoppage

of granular motion.

Fig. 21: Sensory cells in the labyrinth of rabbits. Such cells begin to swell when acetylcholine dissolved in cerebrospinal fluid is applied, as shown in Fig. 22.

A stria vascularis is shown in Fig. 23. The lumina of blood vessels become invisible following application of adrenalin, as shown in Fig. 24.

Fig. 25: Two fibrous layers are distinguishable in the tectorial membrane. Upon application of 10% formol the fibres become more conspicuously visible, but seem unnaturally stiffend. Similar result is given by application of Wittmaack's fixer.

III. b. Otoliths and chemical solutions

Fig. 27: Upon application of ordinary decalcifying solutions, otoliths gradually dissolve, generating carbonic acid gas. Fig. 28 shows them 5 minutes after the application. They disappear completely in 15 minutes, only bubbles being left, as shown in Fig. 29.

Otoliths dissolve in solutions of potassium bichromate and sodium citrate, too.

Fig. 30 shows otoliths before being acted upon by sodium bicarbonate. No change is observable after its application (Fig. 31).

Fig. 32: Otoliths under strong magnification. When subjected to the action of highly diluted 0.1% acetic acid, their edges break away, like worm-eaten leaves (Fig. 33).

Fig. 34: Ditto one hour afterwards.

Fig. 35: Ditto one and a half hour later.

In approximately two hours, only traces are left of the stones (Fig. 36). The above cited modes of change are common to otoliths of rabbits, guinea-pigs and frogs with little divergence.

Conclusion

1. The authors of this paper have fairly succeeded in grasping the living state of the various parts of the membranous labyrinth by utilizing a phase contrast microscope for their examination. These experiments have the advantage in that they can rid themselves of the fixation method and take living cells as their object. Also the time required for the preparation of microscopic slides is cut down to almost nothing.

2. We could herein follow-up the so-called post-mortem degenerations of Corti's organ and arrive at many valuable observations connected with the disintegration, swelling and bubble formation occurring therein.

3. The various cells in the ear-labyrinth showed diverse reactions upon application of several chemicals and medicaments, such as swelling, contraction, stoppage of granular movement etc.

Fig. 1

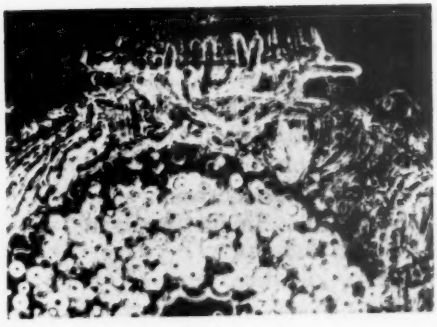


Fig. 2

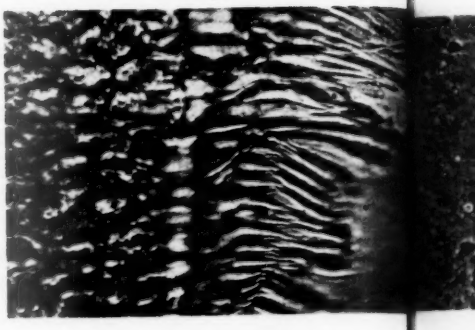


Fig. 3

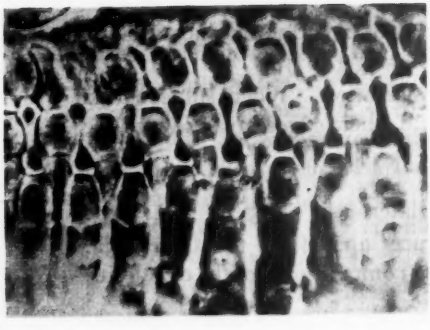


Fig. 4

Fig. 5

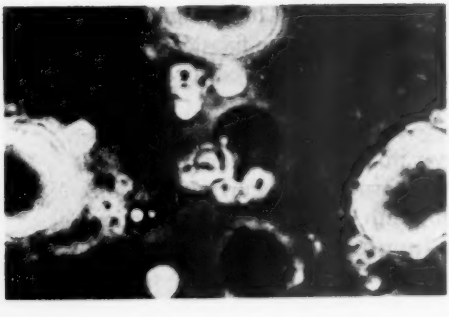


Fig. 6

Fig. 7

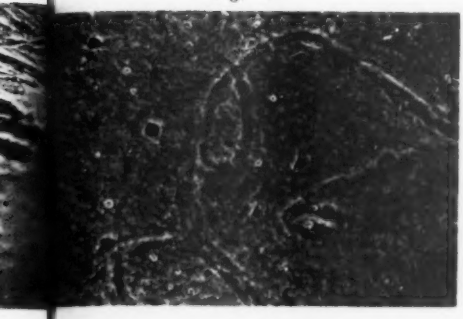


Fig. 8

Fig. 9

Fig. 10

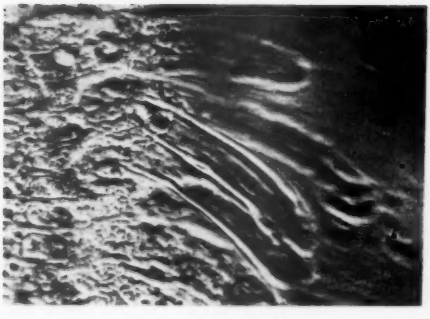


Fig. 11

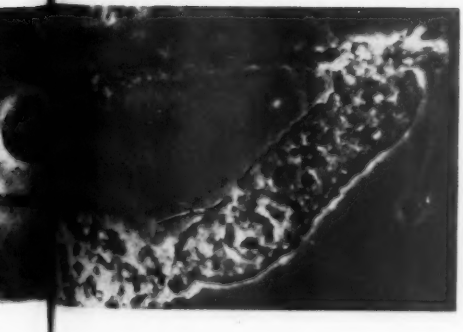


Fig. 12

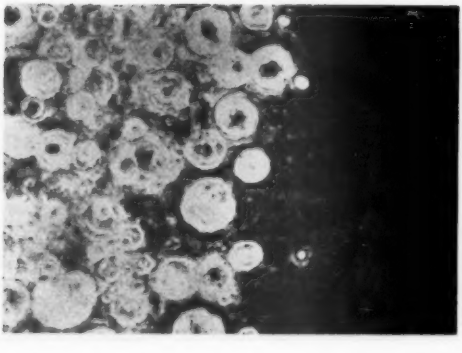
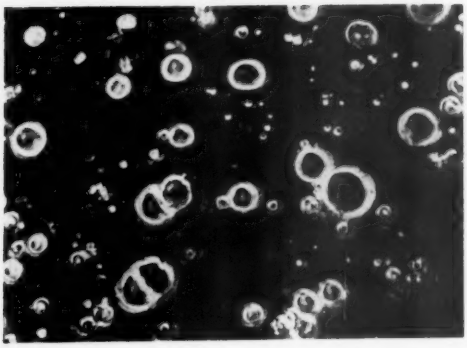


Fig. 13



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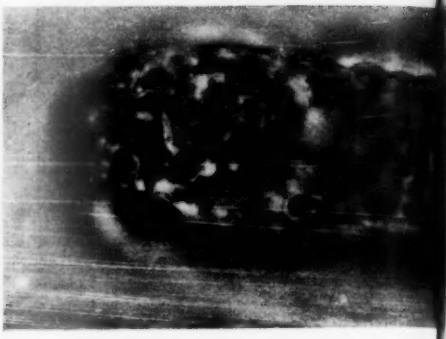


Fig. 15

Fig. 16

Fig. 17

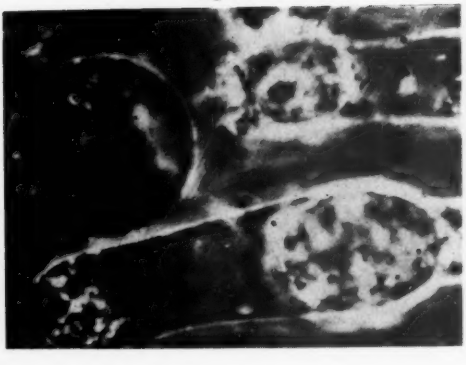


Fig. 18

Fig. 19

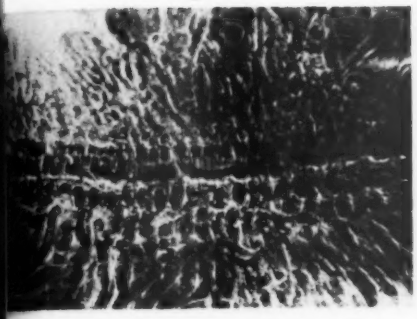
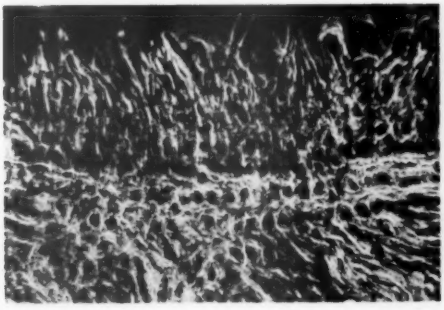


Fig. 20



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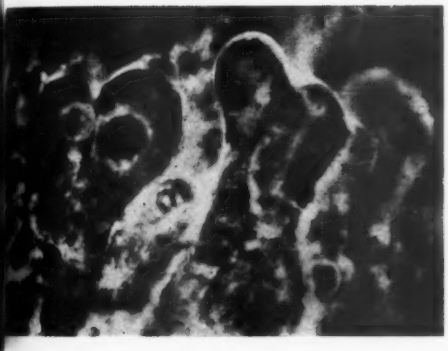

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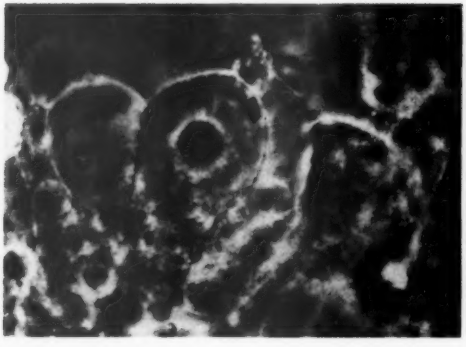

Fig. 23

Fig. 24

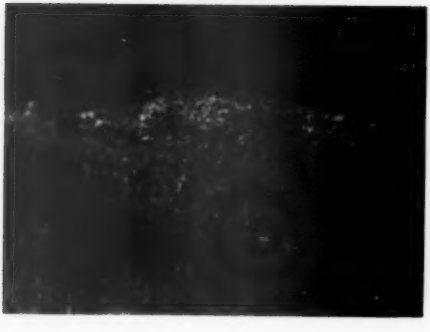

Fig. 25

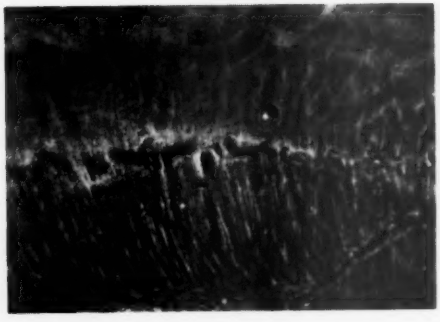

Fig. 26

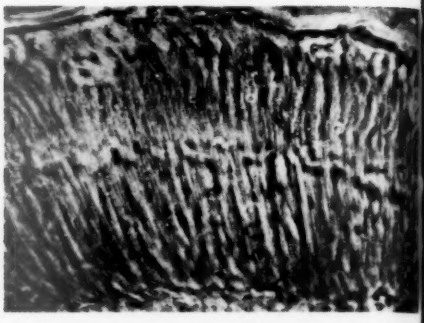

Fig. 27

Fig. 28

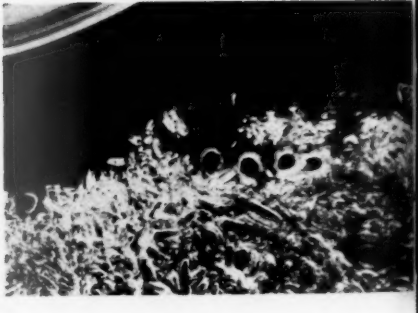

Fig. 29

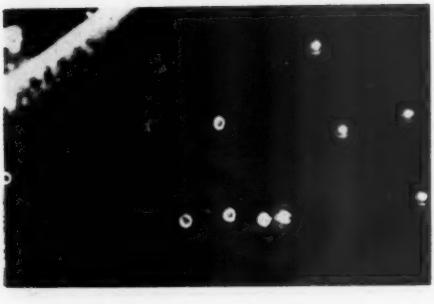

Fig. 30

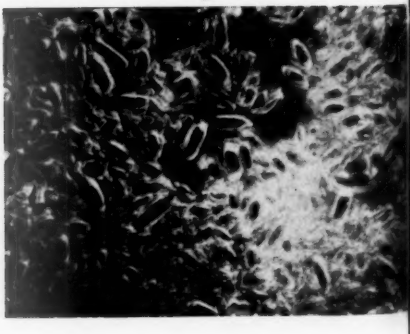

Fig. 31

Fig. 32

Fig. 33

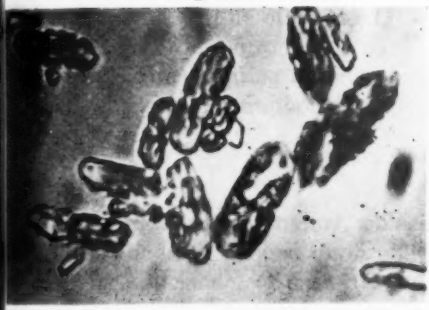

Fig. 34

Fig. 35

Fig. 36

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Preliminary Report on an Electrophoretic Method for Analyzing Liver Proteins

By

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(Received for publication, June 18, 1954)

Two methods, as developed by Luck[1] and by Fishmann[2], are known for estimating the protein fractions of the liver. However, the former method is inconvenient for clinical uses in that the procedure is complicated and a large amount of material is required for examination; in the latter method, on the other hand, the procedure is quite simple, but estimations are a little too rough.

In estimating the liver protein fractions we have devised a method in which the electrophoretic pattern is used, as will be reported in the present paper.

Procedure

During laparotomy, a block weighing 3 to 4 g. is taken from the margin of the liver, divided into small pieces, and pressed to squeeze out blood as far as possible. About 100 mg. of small pieces are taken and weighed, and the protein content is determined by the method of Fishmann.

The remaining liver pieces are ground in a mortar after being well mixed with about 20 cc. of physiological saline. The sample is then centrifuged at 2000 rpm. for about 10 minutes. To the supernatant is added about 5 times as much absolute alcohol to precipitate the protein. The mixture is centrifuged; the sediment is mixed with about 20 cc. of ether and centrifuged, and the sediment thus obtained is dried in a desiccator and used as the test material.

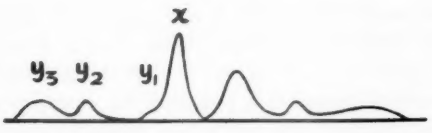
About 300 mg. of the test material (dried material) is weighed, to which about 3 cc. of 1 per cent sodium hydroxide solution is added. After being left for 5 hours or so to dissolve most of the material, the mixture is centrifuged, and the supernatant is dialyzed against a phosphate buffer solution at pH 7.8 for 24 hours or longer. The electrophoretic separation of the proteins of the dialyzed material is performed in the same buffer solution under the following conditions: protein content; 2 per cent; ionic strength; 0.29; 15°C; 100 volts; 15mA.

Electrophoretic pattern of the liver protein

Four representative electrophoretic curves of the liver protein, which were obtained after about 60 minutes of electrophoresis under the above-mentioned conditions, are shown in Figures 1 to 4. It will be seen that the number of the peaks and the shape of each peak are nearly constant, though the height of each peak may be different according to cases.

On the ascending side (left half) of the pattern in Fig. 1 there are found 4 peaks, which will be referred to, for convenience' sake, as x, y_1, y_2, and y_3 in the decreasing order of electrophoretic mobility of the fractions.

Fig. 1



Findings obtained as to each fraction:

Peak y_1 is located close to x, and usually low.

Peak y_2 does not appear in some cases (Figs. 2, 3), and may clearly appear in others (Fig. 1).

Fig. 2

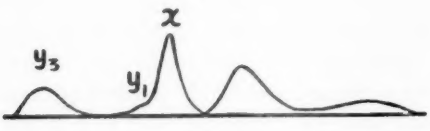


Fig. 3

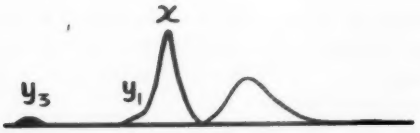
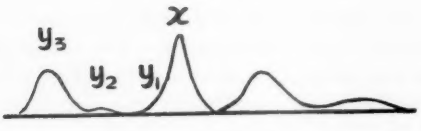


Fig. 4



The height of y_3 is usually of the order seen in Figure 1. The peak may be higher or lower (Figs. 2, 4 or Fig. 3) according to cases; and it may often be quite absent.

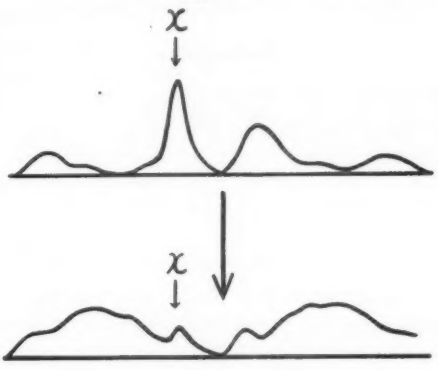
Peak x is usually the highest of the four.

The separation of peaks on the descending boundary (right half of the pattern) was not clear.

Qualitative test for the chemical nature of the peaks of the electrophoretic pattern

For studying the chemical nature of the fractions represented by the peaks of the electrophoretic pattern the following preliminary test was made. To a solution of the test material was added an equal volume of saturated ammonium sulfate solution. The resulting precipitate was put in physiological saline, where it would dissolve mostly. To this saline was added an equal volume of saturated ammonium sulfate, and the resulting precipitate was again dissolved in physiological saline. This process of half-saturation with ammonium sulfate was repeated six times, half-saturation and the last precipitate was dissolved in the saline and submitted to electrophoresis, with the results as shown in Fig. 5. It will be

Fig. 5



seen that peak x almost disappeared, while the peaks of group y were present as before. From this it might possibly be assumed that peak x represented the albumin component of the liver and peaks of group y the globulin. If so, the amounts of the fractions of peaks x and y's as estimated by the weighing method should agree, respectively, with those of the albumin and globulin fractions of the liver as determined by the method of Fishmann. There were found, however, large differences between both series of values, as shown in Table I. As such discrepancies

TABLE I

Comparison of the Amounts of the "seemingly" Albumin and Globulin Fractions separated electrophoretically with the Amounts of the Albumin and Globulin Components determined chemically

	Electrophoresis	Chemical analysis
Albumin	9.0 g/dl	6.0 g/dl
Globulin	3.5 g/dl	6.5 g/dl
A/G	2.6	0.9

were always seen with different test materials, the above test seems insufficient; and finer qualitative tests would be necessary to obtain reliable information as to the chemical nature of the peaks.

Summary and Conclusions

So far as is known, the method of electrophoresis has not yet been applied to the analysis of the liver protein. Attempts have been made to apply the electrophoretic method with some successful, though not best, results. As to the nature of the peaks as seen on the electrophoretic pattern, one of the writers (T.S.) is making investigation.

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Electrophoretic Studies of Liver Components

Studies on Protein Fraction.

Report VIII

By

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(Received for publication, June 18, 1954)

In a series of electrophoretic studies of the liver protein components, first we succeeded in obtaining satisfactory electrophoretic patterns, as repeatedly reported elsewhere[1-4]. In this line of study, no report other than that of Luck[5] is on hand; on the other hand, further investigation has been required at some points of our method. The present paper is to report the results of various examinations of different procedure, comparing electrophoretic patterns obtained by them.

Ideally speaking, the liver extract should be directly used for electrophoretic studies of liver components; but it was too turbid to be used for electrophoresis. Various method, therefore, were tried to eliminate this turbidity.

Experimental

Extraction from Dry Liver Powder

As shown in Table I, a block of fresh liver tissue was ground, and dried to a fine powder. From 300 to 500 mg. of the powder was extracted in 0.9 per cent sodium chloride solution or in a buffer of pH 7.8. The extract was soon dialyzed against a buffer of pH 9.6. Electrophoresis (100 V, 15 mA, 15°C, 60 min.) was performed on the dialyzed extract, with the results as shown in Fig. 1. It will be seen that by the use of either solution for extraction there appeared two peaks during electrophoresis, a peak (x) which overlaps the corresponding peak on the other boundary (descending) and a peak (y) which corresponds to the fraction of smaller electrophoretic mobility. Though transparent, both extracts were of intense yellow-brown colour, which often made it difficult to obtain clear-cut electrophoretic patterns by this method.

TABLE I

Extraction from the Dry Powder of Liver Tissue

Fresh liver (3-4 g.)
↓ Finely ground
Dry powder (300-500 mg.)
↓ 0.9% NaCl, etc., 4-5 cc.
Extract
↓
Dialysis (24 hr. against pH 9.6 buffer)
↓
Electrophoresis
(100V., 15 mA., 15°C)

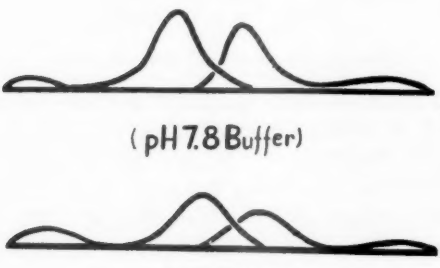

Fig. 1. Electrophoretic patterns of the liver components as extracted from dry liver powder.

High-speed Centrifugation of the Extract of Fresh Liver

As shown in Table II, a block of fresh liver tissue was homogenized in one of the following solutions for extraction 0.9 per cent NaCl, 5.0 per cent NaCl (pH 5.0)[5], and buffers of pH 7.8 and 9.6. Each extract was centrifuged at 15,000 rpm. for 30 minutes, and the supernatant was dialyzed against the buffer of pH 9.6. Electrophoresis was then carried out, with the results as shown in Fig. 2. Both peaks (x, y) were seen in each of the patterns in the four cases, y being lower generally and definite only when the pH 7.8 buffer was used. It was found that even by high-speed centrifugation the turbidity of the extract could not be perfectly eliminated, and clear-cut electrophoretic patterns were not obtained in some cases.

Treatment of the Extract of Fresh Liver with Alcohol

We have succeeded in obtaining a clear-cut electrophoretic patterns

TABLE II

High-speed Centrifugation of the Extract of Fresh Liver

Fresh liver (3-4 g.)
↓ 0.9% NaCl, etc., ca. 20 cc.
Hemogenized
↓
Supernatant — Sediment
↓ 15,000 rpm. 30 min.
Supernatant — Sediment
↓
Dialysis (longer than 24 hr. against pH 9.6 buffer)
↓
Electrophoresis (100V., 15 mA., 15°C)

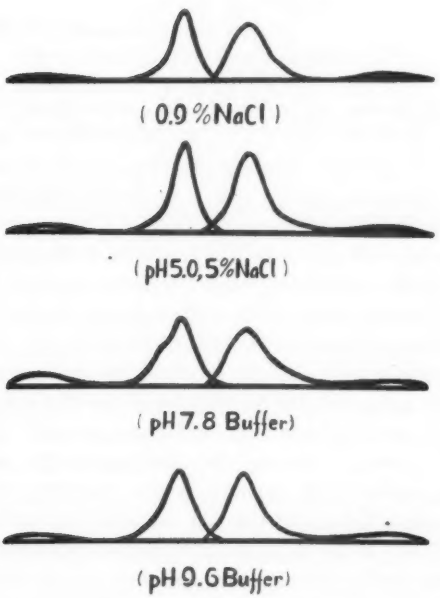

Fig. 2. Electrophoretic patterns of the liver components as treated by high-speed centrifugation.

by procedure outlined in Table III. Until then, various examinations had been made upon the electrophoretic and related procedures, as will be described in the following:

Choice of the solution for extracting fresh liver. In order to choose the

TABLE III

Alcohol Treatment of the Extract of Fresh Liver

Fresh liver (3-4 g.)
↓ 0.9% NaCl, ca. 20 cc.
(Finely ground)
↓
Supernatant | Sediment
↓ 5 vols. of alcohol
Sediment
↓ Ether-treated, then vacuum dried
Test material (200-300 mg.)
↓ 0.5% NaOH 4-5 cc.
Test material solution
↓
Dialysis
↓ (longer than 24 hr, against pH 9.6 buffer)
Electrophoresis (100 v., 15 mA., 15°C)

solution most suitable for extracting finely ground fresh liver, 0.9 per cent NaCl, 5.0 per cent NaCl (pH 5.0), phosphate buffer of pH 7.8, and a carbonate buffer of pH 9.6[6] were tested, electrophoresis being performed in the manner as shown in Table III. As nearly similar electrophoretic patterns were obtained in all the cases (Fig. 3), it was decided to use, for further examination, 0.9 per cent NaCl, which is simplest to prepare. To the supernatant of this extract was added 5 times as much absolute alcohol; the sediment was treated with ether and vacuum-dried; the dry substance thus obtained will be referred to as "test material."

Choice of the solution to be used for dissolving the test material. In order to choose the solution most suitable for dissolving the test material, five NaOH solutions (Fig. 4), and 0.9 per cent NaCl and the pH 7.8 and 9.6 buffers as controls were tested. Among the electrophoretic patterns obtained (the time shown under each pattern is the duration of electrophoresis), those in 0.9 per cent NaCl and the pH 7.8 and 9.6 buffers wree similar to one another: Already in 15 to 20 minutes of electrophoresis there appeared a low overlapping peak, followed by two low gently-sloping peaks, and then by the highest peak of the smallest mobility. It required, however, more than 800 mg. of the test material (corresponding to more than 8 g. of fresh liver) to obtain such patterns.

When 0.1 or 0.2 per cent NaOH was used, the preceding peak was low but clearly visible, the next two peaks were larger and clearer than in the above cases, and the last peak was the largest of the four.

In cases of 0.3 per cent NaOH, the second and third peaks became

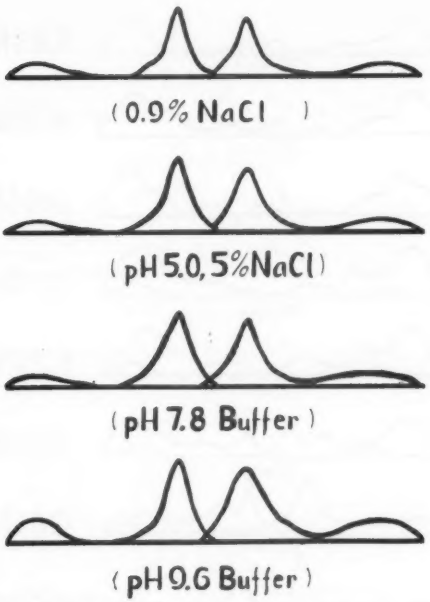

Fig. 3. Different electrophoretic patterns of the liver components extracted from fresh liver with different solutions.

more remarkable, the former being the highest next to the fourth.

In cases of 0.4 per cent NaOH, the preceding peak was still visible in many instances, the second became much higher, the third low and gently-sloped, and the last less in height.

In cases of 0.5 to 1.0 per cent NaOH, the preceding peak disappeared in many instances, and patterns usually, with 4 peaks were obtained as reported in a previous paper[1),4)].

The amount of the test material required to obtain such electrophoretic patterns as mentioned above differed in accordance with the concentration of NaOH in which the material was dissolved: more than 800 mg. for 0.1 per cent NaOH, more than 500 mg. for 0.2 to 0.4 per cent NaOH, and 200 to 300 mg. for 0.5 to 1.0 per cent NaOH.

Which of the electrophoretic patterns represents the nature of the test material most definitely is an important question. It is easily conceivable that decomposition or denaturation of the tissue components might be only slight, if any, in media of weak alkalinity such as the buffers of pH 7.8 and 9.6, but might become more and more significant with increase in the alkalinity. On the other hand, the amount of the test

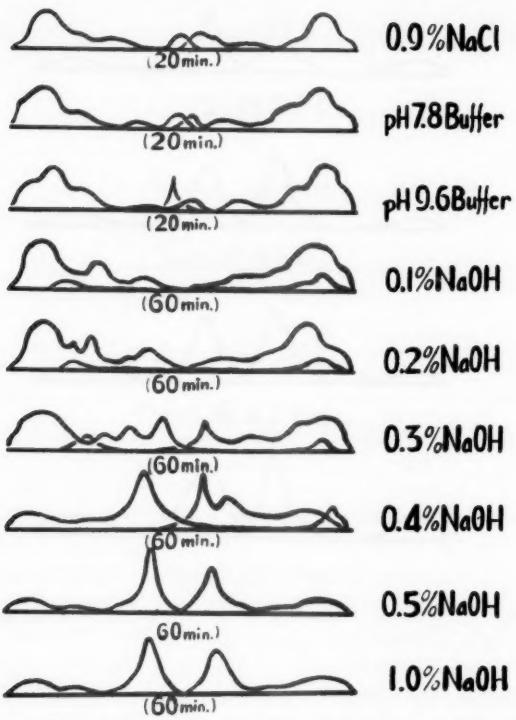

Fig. 4. Different electrophoretic patterns of the liver components, as obtained when the test material was dissolved in various solutions.

material required to obtain clear electrophoretic patterns was inversely proportional to the alkalinity of media, as mentioned above, for example, as small as 200 mg. for 0.5 to 1.0 per cent NaOH. Furthermore, in clinical cases the amount of liver tissue to be taken for examination was limited. Taking all these points into consideration, it was decided to use 0.5 per cent NaOH for dissolving the test material and to take that portion of the material which was dissolved within 20 to 30 minutes.

Choice examination of the buffer to be used for electrophoresis. As buffers for dialysis and electrophoresis of the test material solution the two previously mentioned buffers of pH 7.8 and 9.6 were tested, with the results as shown in Fig. 5. It will be seen that electrophoretic patterns as obtained with the two different buffers are nearly the same in shape, having four peaks, which are referred to as P_1, P_2, C_1, and C_2 (Fig. 6) for the reason to be stated later. The content of each fraction corresponding to each peak

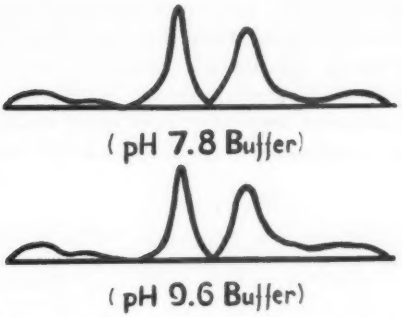

Fig. 5. Electrophoretic patterns of the liver components, as obtained when two different buffers were used for dialysis and electrophoresics.

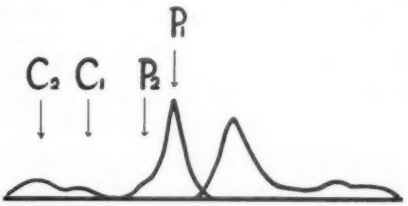

Fig. 6. Designation of the peaks as seen in the electrophoretic pattern.

to both patterns, which was estimated by the weighing, is given in Table IV. It will be found that there was little difference between the corresponding values of each fraction. The buffer of pH 9.6, however, was

TABLE IV

Values of Each Fractions in Both Patterns, as obtained when Two Different Buffers were used for Dialysis and Electrophoresis

	P_1 (%)	P_2 (%)	C_1 (%)	C_2 (%)
pH 7.8	71.4	6.0	4.2	18.4
pH 9.6	71.2	5.8	4.0	19.0

used for further examination, because the use of the buffer of pH 7.8 often caused turbidity during the course of dialysis.

Qualitative tests of the fractions of electrophoretic patterns by treatment with alcohol. The question which of the electrophoretic patterns represents the nature of the liver components more faithfully is still under investigation; but it has been found that if the method of alcohol treatment shown in

Table III is adopted, a clear-cut pattern of constant shape will be obtained with a minimum amount of liver tissue for elctrophoresis. The findings thus far obtained as to the biochemical nature of each fraction separated by the present method of electrophoresis are as follows:

First, several qualitative tests were performed upon the test material (Table III), with the results shown in Table V: the biuret test, Molish's

TABLE V

Results of Qualitative Tests upon "Test Material"

Test	Reaction
Biuret test	(++)
Molish's test	(++)
Test for phosphorus	(++)
Bial's test	(+)
Dische's test	(−)
Neuberg-Saneyosi's test	(±)
Iodine test	(±)

test, and a test for phosphorus were strongly positive; Dische's test[7] was negative; Bial's test[8] was weakly positive; and Neuberg-Saneyosi's test[9] and the iodine test were faintly positive. These results are indicative of the presence of proteins, carbyhydrates, and nucleic acids. Attempts were made to get separate fractions by the process of salting-out, but they were not successful.

An attempt to get separate fractions electrophoretically, however, gave satisfactory results: By the use of the fractionating cell (made by the Hitachi Manufacturing Co.) the test material was separated into two groups, a fraction (P) including peaks P_1 and P_2 and another fraction (C) including peaks C_1 and C_2 (Fig. 7). That the separation had been complete was ascertained by making electrophoresis of each fraction. Qualitative tests were made upon the two fractions, with the results shown in Table VI: In fraction P, the biuret test was strongly positive, Molish's and Bial's tests were weakly positive, and the test for phosphorus was faintly positive. In fraction C, Molish's test was strongly positive, the biuret test was faintly positive, Bial's test and the iodine test were positive. From these results it may be taken that fraction P represented the protein group and fraction C the carbohydrate group.

Method for examining electrophoretic patterns of the alcohol-treated liver material. Electrophoresis was performed upon many samples from normal and diseased livers, and the resulting electrophoretic patterns were examined comparatively. There were found some cases in which clear

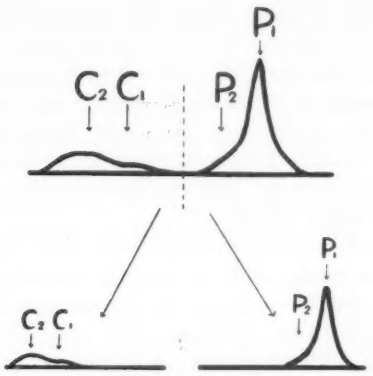

Fig. 7. Electrophoretic patterns of two fractions separated by the use of the fractionating cell.

TABLE VI

Results of Qualitative Tests upon Each Fraction

Test	P_1, P_2 fraction	C_1, C_2 fraction
Biuret test	(卅)	(±)
Molish's	(+)	(卅)
Test for phosphorus	(±)	(+)
Bials test	(+)	(+)
Dische's test	(−)	(−)
Neuberg-Saneyosi's test	(−)	(−)
Iodine test	(−)	(−)

separation of the four peaks (P_1, P_2, C_1, C_2) was impossible. Even in such cases a clear distinction could always be made between the P group (P_1, P_2) and the C group (C_1, C_2). Then, the contents of the P and C fractions can be estimated by the weighing method as in the cases of electrophoretic patterns of serum proteins. The results of this method will be reported elsewhere.

Discussion and Conclusions

Electrophoretic studies of various kinds of tissue, especially those of the liver extract, have recently been made first by Luck, then by Araki.[10] Experimental procedures used by them, however, were so complicated and required so large an amount of liver tissue that they were not suitable for clinical use. In clinical application of the electrophoretic method several difficulties were experienced: For instance, a large amount of liver

tissue could not be taken from patients; the extract often became turbid in the course of dialysis. Various attempts were made to solve these difficulties and to obtain constant and clear-cut electrophoretic patterns, with some satisfactory results as reported partly in previous papers.

Extraction of the dry powder prepared from liver tissue or high-speed centifugation of the extract of fresh liver tissue made it possible to get electrophoretic patterns, but the patterns thus obtained were not very satisfactory in many cases.

As to the alcohol treatment of the fresh liver extract various kinds of examinations were made, with the results showing that the use of 0.5 per cent sodium hydroxide was to be recommended in that clear-cut patterns could constantly be obtained with a small amount of the test material (Table III). These patterns had four peaks, which have been referred to as P_1, P_2, C_1, and C_2.

Several qualitative tests upon the test material dissolved in 0.5 per cent NaOH revealed the presence of proteins, carbohydrates, and nucleic acids. By the use of the fractionating cell in electrophoresis the test material was separated into two fractions: one corresponding to the P peaks (P_1, P_2) and the other to the C yeaks (C_1, C_2). From qualitative tests upon each fraction it may possibly be concluded that the P fraction represented the protein group and the C fraction the carbohydrate group.

Summary

Various tests which were made to get satisfactory electrophoretic patterns of the liver extract are described, and the electrophoretic patterns thus obtained are shown. Clear-cut patterns were constantly obtained with a minimum amount of material when fresh liver tissue was extracted with 0.9 per cent NaCl, treated with alcohol, and dissolved in 0.5 per cent NaOH. The patterns revealed a fraction representing the protein group and a fraction representing the carbohydrate group.

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Studies on the Pathogenetic Microbes in Bone Marrow in Typhoid and Paratyphoid

1st Report

Results of Cultures of Pathogenetic Microbes from Bone Marrow in Typhoid and Paratyphoid

By

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At the present time the bone marrow puncture has been widely adopted not only in hematologic[1)-27)], but also in infectious diseases[28)-49)].

In my studies on the pathogenetic microbes from bone marrow I examined 536 typhoid, 15 paratyphoid A and 109 paratyphoid B cases during the five years 1938—1942.

Of these 660 patients 325 were male and 335 female. The bone marrow cultures of all patients were done regularly once a week by serial cultures following recovery, except in case of death or leaving our hospital for some unavoidable reason

The patients ranged in age from 6 months to 76 years. From one to fifteen bone marrow cultures were made and examined per patient, amounting to 1727 cultures examined in typhoid, 46 in paratyphoid A, and 262 in paratyphoid B, totalling 2035.

Experimental

The bone marrow specimen was taken from the breast bone in the second intercostal space. 3 cc. of bone marrow specimen were put into 5–7 cc. of bile media. After enrichment in bile media the content was painted on the surface of Endo's media and cultivated at 37°C. This procedure was done repeatedly every day until the pathogenetic microbes isolated as colonies, and when the cultures turned out to be negative after 5 day's enrichment in bile media I determined them to be negative. As a control 3cc. of venous blood from the same patient was cultured at the same time in the same way. In addition to this, other materials —stool, urine, pharyngeal mucus—were examined regularly once a week on the Endo's media.

Results

1. *Bone marrow culture on the day of admission and in the course of the illness*

In Table I are listed the percentages of positive findings in bone marrow cultures in 660 cases on the day of admission. The microbes were culturally positive in the typhoid group as follows: bone marrow—87.3%; blood—56%; stool—37.5%; urine—7.8%; Pharyngeal mucus—2.2%. In the Paratyphoid A group: bone marrow—86.7%; in the paratyphoid B group: bone marrow—75.2%.

TABLE I

The Results of the Bone Marrow Cultures on Admission and in the Course of Typhoid and Paratyphoid Cases

Species	No. in which Cultures were made	Period in which cultures were made	Bone Marrow		Blood		Stool		Urine		Pharyngeal mucus	
			No.	%	No.	%	No.	%	No.	%	No.	%
Typhoid	536	Admission	468	87.3	300	56.0	201	37.5	42	7.8	12	2.2
		In the course	476	88.8	344	64.2	318	59.3	112	20.9	34	6.3
Paratyphoid A	15	Admission	13	86.7	5	33.3	5	33.3	1	6.7	0	
		In the course	14	93.3	7	46.7	9	60.0	2	13.3	1	6.7
Paratyphoid B	109	Admission	82	75.2	33	30.6	43	39.5	9	8.3	4	3.7
		In the course	84	77.1	36	33.0	53	48.6	16	14.7	7	6.4
Total	660	Admission	563	85.3	338	51.2	249	37.7	52	7.9	16	2.4
		In the course	574	87.0	387	58.6	380	57.6	130	19.7	42	6.4

I found that the results of the positive findings in the bone marrow were excellent in comparison with those in blood and the patient's other materials.

The percentages of the positive findings in the bone marrow are closely related to the severity of the illness. The clinical course of typhoid is usually more severe than paratyphoid and so the percentages of the positive findings in the bone marrow of typhoid are higher than those of paratyphoid.

2. *Bone marrow culture in every week of the illness*

Table II shows the positive findings of the bone marrow culture, obtained through 2035 examinations. Each case was observed regularly with one culture a week through the course of the disease. The percentage of the positive findings in the bone marrow in typhoid was in the first week of the illness 91.9%, while that in blood was only 76.6%. More-

over, after the second week of the illness the percentage of positive findings in blood decreased rapidly, but in bone marrow culture it decreased slowly. The curves of the percentages of positive cultures in paratyphoid A and B are similar to those seen in typhoid.

TABLE IIa

The Cultural Findings for Bacilli of Bone Marrow in Every Week of the Illness

(Typhoid 536 Cases)

Week of the illness	No. in which culture were made	Positive									
		Bone marrow		Blood		Stool		Urine		Pharyngeal mucus	
		No.	%	No.	%	No.	%	No.	%	No.	%
I	111	102	91.9	85	76.6	34	30.6	6	5.1	1	0.9
II	356	289	81.2	170	47.8	121	34.0	17	4.8	4	1.1
III	403	278	69.0	127	31.5	161	40.0	36	8.9	13	3.2
IV	321	175	54.5	64	19.9	97	30.2	36	11.2	10	3.1
V	214	106	49.5	34	15.9	56	26.2	27	12.6	9	4.2
VI	129	58	45.0	14	10.9	32	24.8	28	21.7	4	3.1
VII	81	30	37.0	10	12.4	16	19.8	23	28.4	2	2.5
VIII	43	19	(44.2)	4	(9.3)	9	(20.9)	13	(30.2)	1	(2.3)
IX	25	12	(48.0)	5	(20.0)	6	(24.0)	6	(24.0)	0	
X	17	10	(58.8)	1	(5.9)	9	(52.9)	6	(35.3)	1	(5.9)
XI	8	5	(62.5)	2	(25.0)	1	(12.5)	1	(12.5)	1	(12.5)
XII	5	4	(80.0)	1	(20.0)	1	(20.0)	1	(20.0)	1	(20.0)
XIII	6	4	(66.7)	0		2	(33.3)	3	(50.0)	1	(16.7)
XIV	4	3	(75.0)	0		3	(75.0)	1	(25.0)	1	(25.0)
XV and after	4	0		0		1	(25.0)	0		1	(25.0)
Total	1727	1095	63.4	517	29.9	549	31.8	204	11.8	50	2.9

In paratyphoid, particularly paratyphoid B, the microbes disappeared from the bone marrow more quickly than in typhoid. The typhoid bacilli existed in the bone marrow for the longest time, the paratyphoid A bacilli for a moderate time, and the paratyphoid B bacilli only for a short time.

In the bone marrow culture there is a little difference between male and female, the bacilli existing in the bone marrow of females a little longer than in males.

Concerning the age of patients the icnidence of the disease and the

TABLE IIb
Paratyphoid

Species	Paratyphoid A						Paratyphoid B					
Cases	15						109					
Positive / Week of the Illness	No. in which Cultures were made	Bone Marrow No. %	Blood No. %	Stool No. %	Urine No. %	Pharyngeal Mucus No. %	No. in which cultures were made	Bone Marrow No. %	Blood No. %	Stool No. %	Urine No. %	Pharyngeal Mucus No. %
I	1	1 100	0	0	0	0	27	26 96.3	15 55.6	12 44.4	2 7.4	0
II	9	8 88.9	4 44.4	3 33.3	0	0	81	54 66.7	16 19.8	36 44.4	6 7.4	4 4.9
III	13	10 76.9	3 23.1	5 38.5	0	0	84	25 29.8	5 6.0	19 22.6	6 7.1	3 3.6
IV	12	5 41.7	1 8.3	0	2 16.7	1 8.3	46	7 15.2	0	11 23.9	7 15.2	2 4.4
V	8	3 37.5	2 25.0	2 25.0	1 12.5	0	15	3 20.0	2 13.3	3 20.0	1 6.7	3 20.0
VI	2	1 50.0	0	0	1 50.0	0	4	0	0	1 25.0	1 25.0	0
VII	1	0	0	0		0	4	1 25.0	0	2 50.0	0	1 25.0
VIII					0		1	0	0	1 100	1 100	1 100
Total	46	28 60.9	10 21.7	10 21.7	4 8.7	1 2.2	262	116 44.3	38 14.5	85 32.4	24 9.2	14 5.3

TABLE III

The Results of Bone Marrow Cultures in Every Age Group

(Typhoid 536 Cases)

Age Groups	Under 5 Years					6～10 Years					11～15 Years					16～20 Years				
Cases	15					87					81					109				
Death and (Death rate)		2. (8.7%)					2. (2.3%)					2. (2.1%)					15. (13.8%)			
Week of the illness	No. in which cultures were made	Bone Marrow		Blood		No. in which cultures were made	Bone Marrow		Blood		No. in which cultures were made	Bone Marrow		Blood		No. in which cultures were made	Bone Marrow		Blood	
		No.	%	No.	%		No.	%	No.	%		No.	%	No.	%		No.	%	No.	%
I	8	8	100.0	6	75.0	30	27	90.0	25	83.3	20	18	90.0	14	70.0	20	20	100.0	18	90.0
II	14	12	85.7	8	57.1	61	49	80.3	23	37.7	65	51	78.5	33	50.8	75	66	88.0	41	54.7
III	15	10	66.7	5	33.3	63	35	55.6	14	22.2	66	48	72.7	15	22.7	87	56	64.4	28	32.2
IV	9	6	66.7	0		51	22	43.1	4	7.8	49	24	49.0	5	10.2	69	37	53.6	13	18.8
V	8	3	37.5	0		32	13	40.6	3	9.4	29	9	31.0	2	6.9	44	21	47.7	3	6.8
VI	3	1	33.3	0		16	6	37.5	2	12.5	10	6	(60.0)	2	(20.0)	28	11	39.3	3	10.7
VII	1	0		0		8	3	37.5	0		9	2	(22.2)	0		15	6	(40.0)	2	(13.3)
VIII						3	0		0		6	3	(50.0)	1	(16.7)	8	4	(50.0)	0	
IX											3	1	(33.3)	1	(33.3)	5	3	(60.0)	1	(20.0)
X						1	0		0		1	0		0		3	1	(33.3)	0	
XI																1	0		0	
XII																1	0		0	
XIII						1	1	(100.0)	0							1	0		0	
XIV						1	0		0											
XV						1	0		0											
Total	58	40	69.0	19	32.8	268	156	58.2	71	26.5	258	162	63.0	73	28.3	357	225	63.0	109	30.5

Age groups	21～30 Years					31～40 Years					41～50 Years					51 Years and over				
Cases	98					67					40					31				
Death and (Death rate)		12. (12.2%)					11. (16.4%)					4. (10%)					7. (22.6%)			
Week of the illness	No. in which cultures were made	Bone Marrow		Blood		No. in which cultures were made	Bone Marrow		Blood		No. in which cultures were made	Bone Marrow		Blood		No. in which cultures were made	Bone Marrow		Blood	
		No.	%	No.	%		No.	%	No.	%		No.	%	No.	%		No.	%	No.	%
I	12	10	83.3	7	58.3	14	12	85.7	11	78.6	2	2	100.0	2	100.0	5	5	100.0	2	40.0
II	58	46	79.3	29	50.0	43	30	69.8	16	37.2	22	19	86.4	11	50.0	18	16	88.9	9	50.0
III	75	50	66.7	27	36.0	41	30	73.2	10	24.4	35	29	82.9	15	42.9	21	20	95.2	13	61.9
IV	57	33	57.9	16	28.1	35	20	57.1	12	34.3	29	15	51.7	9	31.0	21	18	85.7	5	23.8
V	43	23	53.5	8	18.6	24	16	66.7	9	37.5	18	12	66.7	4	22.2	16	9	56.3	4	25.0
VI	29	11	37.9	1	3.5	15	9	60.0	3	20.0	16	8	50.0	3	18.8	12	6	50.0	0	0
VII	13	3	23.1	1	7.7	14	9	64.3	3	21.4	14	6	42.9	3	21.4	7	1	14.3	1	14.3
VIII	7	2	(28.6)	0		10	5	(50.0)	2	(20.0)	7	4	(57.1)	0		2	1	(50.0)	1	(50.0)
IV	5	2	(40.0)	1	(20.0)	7	4	(57.1)	2	(28.6)	4	1	(25.0)	0		1	1	(100.0)	1	(100.0)
X	3	1	(33.3)	0		4	3	(75.0)	1	(25.0)	3	3	(100.0)	0		2	2	(100.0)	0	
XI	1	1	(100.0)	0		3	2	(66.7)	2	(66.7)	1	0		0		2	2	(100.0)	0	
XII						1	1	(100.0)	0		2	2	(100.0)	1	(50.0)	1	1	(100.0)	0	
XIII						1	1	(100.0)	0		2	1	(50.0)	0		1	1	(100.0)	0	
XIV						1	1	(100.0)	0		1	1	(100.0)	0		1	1	(100.0)	0	
XV	1	0				2	0		0											
Total	304	182	59.9	90	29.6	215	143	66.5	71	33.0	156	103	66.0	48	30.8	110	84	76.4	36	32.7

death rate are shown in Table III. The younger age groups under 5 years frequently showed serious symptoms and tended to have a higher death rate, while the 6—15 age groups showed the lowest. There is a close relation between the severity of these diseases and the age of the patients. —the mortality rate increased in proportion to their age. The duration of existence of the bacilli in the bone marrow was almost parallel to the patient's age. In the older age groups they existed in the bone marrow for a longer time than in the younger age groups.

3. *Content of the bacilli in bone marrow*

Table IV indicates the number of days which were required for enrichment of the bacilli in bile media. The bone marrow cultures showed excellent results in comparieon to that of the blood. It is very valuable for an accurate diagnosis in the early stage of the illness, that the results of the bone marrow cultures were found to be positive within 24 hours in the first week of the illness, while in that time the Widal's reaction usually turned out to be negative and other symptoms and physi-

TABLE IVa

The Number of Days which were required for Enrichment of the Bacilli in Bile Media

(Typhoid 536 Cases)

Week of the Illness	No. of Cases	Bone Marrow						Blood					
		Enrichment (days)						Enrichment (days)					
		1	2	3	4	5	Sum	1	2	3	4	5	Sum
I	111	98	3		1		102	68	10	5	2		85
II	356	270	11	6	2		289	110	40	14	4	2	170
III	402	240	25	11	2		278	67	31	15	12	2	127
IV	321	148	21	4	1	1	175	41	12	5	4	2	64
V	214	89	14	2	1		106	21	5	5	2	1	34
VI	129	48	9	1			58	7	6		1		14
VII	81	24	4	1	1		30	6	3	1			10
VIII	43	17	2				19	3		1			4
IX	25	9	2	1			12	1	1	2	1		5
X	17	5	4		1		10		1				1
XI	8	3	1	1			5	1	1				2
XII	5	3	1				4		1				1
XIII	6	4					4						0
XIV	4	2			1		3						0
XV	4						0						0
Total	1727	960	97	27	10	1	1095	325	111	48	26	7	517

TABLE IVb

Paratyphoid A and B

(124 Cases)

Week of the Illness	Number of Cases	Bone Marrow						Blood					
		Enrichment (days)						Enrichment (days)					
		1	2	3	4	5	Sum	1	2	3	4	5	Sum
I	28	27					27	10	1	2	2		15
II	90	54	6	2			62	11	4	4	1		20
III	97	23	10	2			35	4	1	3			8
IV	58	8	3	1			12	1					1
V	23	5	1				6	2	2				4
VI	6	1					1						0
VII	5	1					1						0
VIII	1						0						0
Total	308	119	20	5			144	28	8	9	3		48

cal examinations showed nothing characteristics.

As mentioned above, the bone marrow cultures show positive results in higher percentage after shorter enrichment in bile media than the blood culture, but there were a few cases, in which the blood culture was exceptionally superior to the bone marrow culture. In 2035 examinations I found 8 cases (0.4%), in which the blood culture was found to be positive and that of bone marrow turned out to be negative, and there were 3 cases (0.15%), in which the pathogenetic microbes from the bone marrow needed more time for enrichment than those from the blood itself.

As shown in Table V, the duration of enrichment of the bacilli in bile media, in order to isolate them on Endo's media, was observed in 12 cases (7 typhoid, 2 paratyphoid A and 3 paratyphoid B cases). In some of these cases the bacilli were isolated by placing them on Endo's media without enrichment in bile media, but in many of these cases they were first isolated after enrichment in bile media for 6—12 hours. Furthermore I tried to discover how many bacilli were contained in 1 cc. of bone marrow as well as blood. 1 cc. of bone marrow and blood were thoroughly mixed at 45C° with liquefied agar and cultured in a Petri plate in order to count the number of the bacilli as colonies.

Table VI shows that the bacilli were isolated more rapidly and in a much larger number of colonies from bone marrow than from blood. The colonies of bacilli increased to a maximum of more than 700 on the usual gelatine media, into which 1 cc. of bone marrow had been mixed,

TABLE V

The Number of Hours, in Which the Bacilli were Isolated from Bone Marrow and Blood by Means of Enrichment in Bile Media

Name	Age	Day of the illness	Culture							Severity of Case	Comment
			Materials	Direct	after Enrichment						
					2°	4°	6°	12°	24°		
I.F.	40	14th	B.M.	−	−	−	+	++	###	Moderate	Ty.
			Blood	−	−	−	−	−	−		
N.S.	11	9th	B.M.	−	+	++	###	###	###	Moderate	Ty.
			Blood	−	−	+	++	###	###		
M.Y.	16	6th	B.M.	−	−	+	++	###	###	Moderate	Ty.
			Blood	−	−	−	+	++	++		
K.M.	18	8th	B.M.	−	−	−	−	+	++	Severe	Ty.
			Blood	−	−	−	−	−	+		
S.T.	65	11th	B.M.	+	++	###	###	###	###	Death	Ty.
			Blood	−	−	−	−	+	++		
N.A.	18	7th	B.M.	+	+	++	###	###	###	Moderate	Ty.
			Blood	−	−	−	+	++	###		
K.H.	13	17th	B.M.	−	−	−	+	++	###	Mild	Ty.
			Blood	−	−	−	−	−	−		
S.S.	22	12th	B.M.	−	−	−	−	+	++	Moderate	P.A.
			Blood	−	−	−	−	−	+		
K.T.	24	15th	B.M.	−	−	−	+	++	###	Mild	P.A.
			Blood	−	−	−	−	−	−		
K.N.	40	8th	B.M.	−	−	−	−	−	+	Moderate	P.B.
			Blood	−	−	−	−	−	−		
S.K.	10	7th	B.M.	−	+	++	###	###	###	Moderate	P.B.
			Blood	−	−	−	−	−	−		
T.K.	13	6th	B.M.	−	−	−	−	+	++	Mild	P.B.
			Blood	−	−	−	−	−	−		

while the colonies in the blood culture were found to be only one-fifth or one-tenth of the number in the bone marrow culture.

In a large number of cases it was usually noted that the blood cultures were negative, when the bacilli in the bone marrow culture developed into as many as 20 colonies.

In the cases of recurrences, a large number of colonies of the bacilli had been cultured from the bone marrow when the blood culture remained still negative.

CONCLUSIONS

1. In typhoid and paratyphoid, results of the examination of pathogenetic microbes in bone marrow showed a high percentage, from the onset through the entire course of the illness, as compared with those in blood,

TABLE VI

The Number of Bacilli Which were Cultivated from 1 cc. Sternal Marrow and Circulated Venous Blood

No.	Name	Age	Days of The illness	No. of Bacilli in I_{cc}.		Comment
				Bone Marrow	Blood	
T1	T.H.	14	7 th	128	72	Moderate
			14 th	68	0	Severe
			21 th	2	0	
			28 th	0	0	
T2	A.H.	28	13 th	720	126	Letal
			20 th	227	7	
			27 th	108	64	
T3	N.M.	24	10 th	182	94	Severe
			17 th	128	0	
			24 th	6	0	
			31 th	4	0	
			38 th	2	0	
			45 th	0	0	
P.A.	I.S.	26	12 th	22	0	Mild
			19 th	15	0	
			26 th	0	0	
P.B.	K.K.	40	6 th	46	4	Mild
			13 th	17	0	
			20 th	0	0	

stool, urine and pharyngeal mucus.

2. The positive percentages of cultural findings of the bacilli in bone marrow were at a maximum during the first week, and then the curve of the positive findings dropped during the later course of the illness, but more slowly than in blood cultures. The positive findings from bone marrow were after the fourth week of typhoid 3–10 times higher, and after the third week of paratyphoid A and B 3–4 times higher than those of blood cultures.

3. The curve of positive findings of the bacilli in bone marrow in typhoid cases dropped more slowly than that of paratyphoid.

4. The bacilli in bone marrow were closely related to the ages of the patients, the bacilli existing longer in the bone marrow of older patients than of younger ones.

5. The bone marrow bacilli were cultivated in a far shorter time of

enrichment than those in blood, and without exception more bacilli were discovered in an amount of bone marrow than in the same amount of blood.

6. The existence of the bacilli in bone marrow was never directly parallel to the bacilli in stool, urine and pharyngeal mucus.

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Studies on the Pathogenetic Microbes in Bone Marrow in Typhoid and Paratyphoid

2nd Report

The Relation between the Findings of Pathogenetic Microbes in Bone Marrow and Clinical Symptoms of Typhoid and Paratyphoid

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In the same cases, which were discussed in the previous report[1], the relation was observed between the findings of pathogenetic microbes in bone marrow and the clinical symptoms of typhoid and paratyphoid.

1. *Relation between the findings of pathogenetic microbes in bone marrow and the severity of the clinical course*

According to the severity of the clinical symptoms, all the cases were divided into 4 groups: fatal, severe, moderately severe and mild. As the observations show, the more severe the clinical symptoms of the cases were, the higher were the percentages of positive findings of the bacilli in bone marrow, as shown in Table I.

In the fatal cases of typhoid, the positive findings of the bacilli in bone marrow on the very day of admission to our hospital were 98.2%, and during the course of the illness increased to 100%, but in the mild cases they were only 50.7% on the day of admission and 52.1% during illness. On the other hand, the positive findings from blood cultures were only 9.9% on admission, increasing to 12.7% during illness of the mild cases. Therefore the positive results of the bone marrow culture were 5 times higher than the blood culture.

2. *Relation between the findings of the bacilli in bone marrow and the fever*

Concerning the fever period of illness the higher the fever rose, the higher became the percentage of the positive findings of the bacilli in bone marrow, and in the course of fever periods the positive percentage gradually decreased, but very slowly in comparison with the blood cultures even at the same temperature, as the Table II presented.

In general, a younger person is better able to react with fever against bacterial infection than an older one. Therefore, in this case, at the same

TABLE I

The Relation Between the Findings of Pathogenetic Microbes in Bone Marrow and Clinical Symptoms

Species		Typhoid					Paratyphoid A					Paratyphoid B				
Group of Cases	Stage of Cultures	No. of Cases	Bone marrow		Blood		No. of Cases	Bone marrow		Blood		No. of Cases	Bone marrow		Blood	
			No.	%	No.	%		No.	%	No.	%		No.	%	No.	%
Latel	Admission	55	54	98.2	42	76.4										
	During the course		55	100	50	90.9										
Severe	Admission	97	94	96.9	79	81.5	1	1	100	0		4	3	75	2	50
	During the course		94	96.9	88	90.7		1	100	0			4	100	3	75
Moderately Severe	Admission	313	284	90.7	172	55.0	8	7	87.5	4	50.0	48	44	91.7	19	39.6
	During the course		290	92.7	197	62.9		8	100	5	62.5		45	93.8	20	41.7
Mild	Admission	71	36	50.7	7	9.9	6	5	83.3	1	16.7	57	35	61.4	12	21.1
	During the course		37	52.1	9	12.7		5	83.3	2	33.3		35	61.4	13	22.8
Total	Admission	536	468	87.3	300	56.0	15	13	86.7	5	33.3	109	82	75.2	33	30.6
	During the course		476	88.8	344	64.2		14	93.3	7	46.7		84	77.1	36	33.0

TABLE II

The Relation Between the Findings of Pathogenetic Microbes in Bone Marrow and Fever Periods and Temperature

Fever period	Temperature °C	Typhoid					Paratyphoid A					Paratyphoid B				
		No. of cases which cultures were made	Bone marrow		Blood		No. of cases which cultures were made	Bone marrow		Blood		No. of cases which cultures were made	Bone marrow		Blood	
			No.	%	No.	%		No.	%	No.	%		No.	%	No.	%
Fever rising	37.0~37.9	13	13	100	4	30.8	1	1	100	1	100	1	1	100	0	
	38.0~38.9	30	30	100	20	66.7	1	1	100	0		1	1	100	1	100
	39.0~39.9	46	46	100	34	73.9						3	3	100	2	66.7
	Higher than 40.0	5	5	100	5	100										
Acme	38.0~38.9	114	108	94.7	67	58.8	2	2	100	2	100	12	11	91.7	4	33.3
	39.0~39.9	354	340	96.1	217	61.3	4	4	100	2	50	35	32	91.4	14	40.0
	Higher than 40.0	104	102	98.1	78	75.0						2	1	50	1	50
Fever abaiting	37.0~37.9	268	112	41.8	17	6.3	7	5	71.4	1	14.3	45	18	40	4	8.9
	38.0~38.9	296	165	55.7	36	12.2	12	7	58.3	2	16.7	53	29	54.7	7	13.2
	39.0~39.9	150	100	66.7	26	17.3	1	1	100	1	100	26	17	65.4	5	19.2
	Higher than 40.0	25	11	44.0	3	12.0	1	1	100	1	100	1	0		0	
Fever free	Lower than 37.0	322	63	19.6	10	3.1	17	6	35.3	0		83	3	3.6	0	
Total	Fever rising	94	94	100	63	67.0	2	2	100	1	50	5	5	100	3	60
	Acme	572	550	96.2	362	63.3	6	6	100	4	66.7	49	44	89.8	19	38.8
	Fever abating	739	388	52.5	82	11.1	21	14	66.7	5	23.8	125	64	51.2	16	12.8
	Reconverlescent	322	63	19.6	10	3.1	17	6	35.3	0		83	3	3.6	0	

temperature and during the same fever period, the older the patients were the higher were the percentages of the positive cultures, and in some of these cases of older patients, the bacilli were frequently found in bone marrow even in the fever free period (Table II).

3. *Relation between the findings of the bacilli in bone marrow and the blood*

The relation between the Widal's reaction and results of blood and bone marrow culture was described in Fig. 1. As Fig. 1 shows, the Widal's reaction presented nothing characteristics at the onset of the ilness, but became positive during the second week and reached its maximal height at about the 4th to 6th week, and then decreased rather gradually. During recovery the Widal's reaction remained in the majority of cases in titer between 1 :500 and 1 :1000. Fig. 1 shows strkingly that the positive results of bone marrow culture are inversely proportionate to the titer of the Widal's reaction.

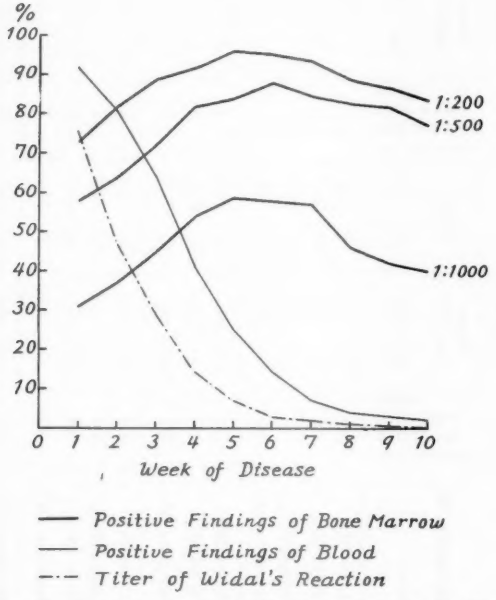

Fig. 1. The Relation Between the Development of the Bacilli in Bone Marrow and Widal's Reactions in 536 Typhoid Cases.

4. *Relation between several symptoms and the bone marrow cultures*

The bone marrow cultures had no relation to the blood pictures. At the onset of the illness the blood sedimentation rate was either normal or only slightly elevated, but during illness it showed a decided increase and

reached its maximal height in about the 5th or 6th week, and returned to normal 3 weeks after the fever free period. The positive bone marrow cultures are inversely proportionate to the sedimentation rate.

Some cases, in which the sedimentation rate remained within normal, in spite of the seriousness of their general features, showed severe involvement or serious prognosis.

There is no intimate relation between the presence of parasite eggs, particularly round worms, and the positive bone marrow culture.

Concerning the urine in the first week, the diazo-reaction was positive in 55% and albuminuria was present in 66% of all the cases. The curves of diazo-reaction and albuminuria run parallel to that of the positive bone marrow cultures, and after the third week these reactions gradually disappeared (Fig. 2, Table III).

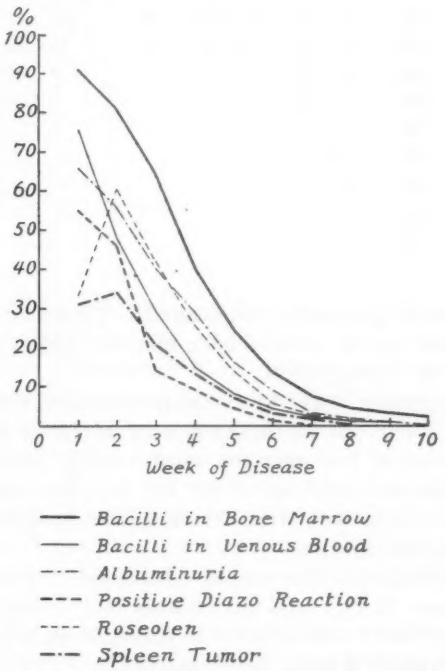

Fig. 2. The relation between the development of the bacilli in bone marrow and enlargement of spleen, appearance of roseolen, diazo-reaction and albuminuria in 536 typhoid cases.

In our cases roseola appeared toward the end of the first week in 32.4%, and increased to a maximum of 60.2% in the second week, but

TABLE

The Development of Chief Symptoms; Splenomegaly, Roseola,

Species	Typhoid											
Symptom / Week of the illness	No. of cases	Splenomegaly		Roseola		Diazo-reaction		Albminuria		No. of cases	Splenomegaly	
		No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%		No. of cases	%
I	111	35	31.5	36	32.4	61	55.0	67	66.4	1	0	
II	357	122	34.2	215	60.2	164	45.9	201	56.3	8	1	12.5
III	432	87	20.2	182	42.1	64	14.9	175	40.5	14	1	7.1
IV	435	60	13.8	117	26.9	39	9.0	129	29.7	15	0	
V	441	29	6.6	58	13.2	16	3.6	71	16.1	15	0	
VI	433	10	2.3	22	5.1	4	0.9	39	9.0	15	0	
VII	431	8	1.9	11	2.6	0		16	3.9			
VIII	432	3	0.7	2	0.5			14	3.5			
IX	432	2	0.5	0				3	0.7			
X	435	2	0.5					2	0.5			
XI	432	0						0				
XII	433	0										
XIII	434	0										
XIV	435	0										
XV	435	0										

after the third week gradually disappeared. Therefore the roseola rash did not run parallel to the positive bone marrow culture in the first week, but did so after the second week.

Spleen enlargement could be recognized toward the end of the first week in 31.5%. The spleen enlarged very much in the second week in 34.2% of the cases, but after the third week it gradually became so much smaller that we could no longer feel it. The rate of recognition of the spleen as a tumor was, after the second week, almost parallel to the positive bone marrow cultures.

5. *Relation between the bone marrow cultures and recurrences and revivals*

29 recurrences (5.4%) and 22 revivals (4.1%) out of 536 typhoid cases, 1 recurrence and 1 revival out of 15 paratyphoid A cases and 1 revival out of 109 paratyphoid B cases were observed.

It is of interest that positive bone marrow cultures were obtained in these recurrences of typhoid cases in 96.6%, but positive blood cultures in only 37.9%. In the revivals the bone marrow culture showed positive findings of bacilli in every case of typhoid and paratyphoid, but in the blood culture positive findings were obtained in only 72.8%. In these

III

Diazo-Reaction and Albuminuria in Every Week

Paratyphoid A					Paratyphoid B								
Roseola		Diazo-reaction		Albumi-nuria		No. of cases	Splenome-galy		Roseola		Diazo-reaction		Albumi-nuria	
No. of cases	%	No. of cases	%	No. of cases	%		No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%
0		0		1	100	27	3	11.1	6	22.2	14	51.9	9	33.3
4	50	3	37.5	5	62.5	83	20	24.1	31	37.4	26	31.3	18	21.7
4	28.6	1	7.1	2	14.3	101	13	12.9	15	14.9	5	4.9	8	7.9
0		0		0		106	4	3.8	5	4.7	2	1.9	5	4.7
						105	1	1.0	0		0		0	
						102	0						2	0.9
						104	0						2	1.8
						104	0						0	

cases the bacilli in excreta, urine and pharyngeal mucus were very rare.

It is very important to note the fact that in patients who were recovering and whose temperature had decreased to normal or showed irregularly slight rises, and whose blood culture had already proved negative, with however only the bone marrow culture yet positive, it has been frequently found that there were later recurrences or revivals.

In fact positive cultures can not usually be obtained from only 3 cc. of blood at the time of fever remission, particularly during the fever free period. The bacilli can be cultured from the circulating blood as soon as they have reached the blood vessels via the lymphatics and have increased enough.

It is noticeable in recurrences and revivals that the bacilli were cultured early from bone marrow of patients whose blood cultures were negative.

6. *Relation between the bone marrow cultures and vaccination*

How does vaccination against typhoid and paratyphoid influence the results of the bone marrow culture? All the cases were divided into 2 groups: vaccinated and non-vaccinated. The positive findings of the

blood culture were strikingly low in the group of vaccinated cases, because perhaps these vaccinated cases remained in a mild stage.

On the contrary, in such cases the bone marrow cultures frequently showed positive, because so many bacilli exsisted in the bone marrow even in mild cases.

In short, the influence of vaccination was scarcely found in the positive findings of the bone marrow culture.

Conclusions

1. In the bone marrow culture the percentage of the positive findings and their duration are in proportion to the severity of the cases. As regards the differences between the percentages of the positive cultures of bone marrow and blood, the milder the clinical symptoms of the cases are, the greater are the differences in the positive findings of both cultures.

2. The percentage of the positive bone marrow culture is high at the beginning of the fever rising period. During the febrile period the differences between the positive cultures of bone marrow and blood gradually become greater.

In the same febrile period the higher the temperature of the patients rises, the higher is the percentage of the positive findings of the bone marrow cultures.

Furthermore, in the same febrile period and at the same temperature, the older the age of the patients is, the higher is the percentage of the positive finding of the bone marrow culture.

3. Positive findings of the bone marrow culture showed the highest percentage at the onset of the illness before any agglutinins are produced, and in the course of the illness the curve of the positive findings of the bone marrow culture gradually dropped. The agglutinations are usually elevated toward the end of the second week of the illness, therefore the curve of the positive findings of the bone marrow culture is inversely proportioned to that of the titer of agglutination.

4. Positive findings of the bone marrow cultures are inversely proportionate to the sedimentation rate of blood, and parallel to the diazo-reaction and albuminuria, but they have no intimate relation to the blood picture.

5. The curves of appearance of roseola and spleen tumor run, after the second week, parallel to those of the positive findings of the bone marrow culture.

6. In recurrences and revivals bacilli from bone marrow are cultured earlier and more plentifully than those from blood.

7. Results of the bone marrow culture are scarcely influenced by vaccination, therefore the bone marrow culture is of practical value in making diagnosis of typhoid and paratyphoid among those who have been vaccinated.

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The Tohoku Journal of Experimental Medicine, Vol. 62, No. 2, 1955

Studies on Pathogenetic Microbes in Bone Marrow in Typhoid and Paratyphoid

3rd Report

The Development of Pathogenetic Microbes in Bone Marrow and the Clinical Significance of Bone Marrow Punctures

By

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It is very interesting to know how typohid and paratyphoid bacilli develop in the body of a human being, particularly in the bone marrow. The bacilli are transmitted from person to person through the medium of stool, urine, sputum, pus and human milk. They usually enter the body from these media through the mouth. They establish themselves and grow in the enteric tract. After the incubation period they enter the blood stream and circulate through the entire body, causing fever. But in the very early satge of the illness they can with difficulty be cultured from the circulating blood of 1–3 cc., which is usually used in practice. On the contrary the bone marrow is rich in capillary vessels and reticulo-endothelial tissues. By its filtering and retaining power it arrests the numerous bacilli in itself, in the early stage of the illness.

1. *Development of pathogenetic microbes in bone marrow*

In 111 typhoid cases, which were observed in the first week, I found only one case, in which on the fourth day after the beginning of the illness the bone marrow culture was negative, but the blood culture showed positive finding. In these examinations, the bone marrow culture is always superior to blood culture.

I have experienced one case, in which bone marrow was cultured in the 24th hour after the beginning of typhoid, and observed at the same time a reciprocal relation of positive findings between bone marrow and blood, stool, urine and pharyngeal mucus.

That is (Table I): K. E., (age 62), farmer's wife. Fever free on the 77th day, as a bacilli carrier in bone marrow she was discharged on the 93rd day of the illness. It is remarkable in this case that the 3 cc. blood culture showed positive finding only in the first week after 24 hours

TABLE

Case of K. E.

Severe, Begining: June 15, 1942; Discharged, September 15, as a Bone

Day of the illness		2	4	9	16	23	31
Week of the illness		I	I	II	III	IV	V
Culture	Bone marrow	++++$_1$	++++$_1$	+++$_1$	++++$_1$	+++$_1$	+++$_1$
	Blood	−	++++$_1$	+$_2$	−	−	−
	Stool	−	+	−	+	−	−
	Urine	−	−	−	−	−	−
	Pharyngeal mucus	−	−	−	−	−	−
Widal's reaction		T − A − B −	T 200 A − B −	T 500 A − B 100	T 500 A − B 100	T 1000 A − B 100	T 500 A − B −
Splenomegaly		−	+	−	−	−	−
Roseola		−	−	+	+	−	−
Diazo-reaction		−	−	−	−	−	−
Albuminuria (Urine)		−	−	−	−	−	±
Temperature °C		38.5	38.5	38.4	38.2	38.3	38.3
Comment		24 hours after the biginning	Decubitus	Entero-rrhagia	Suppura-tion of her Neck		

enrichment, and in the second and ninth week after enrichment of 2 days in bile medium, while the bone marrow culture was always positive and many colonies were cultured after 24 hours enrichment.

Table II shows the typhoid and paratyphoid cases, in which bone marrow was cultured within 7 days after the onset of the illness.

The highest percentage of the positive bone marrow cultures appeared at the beginning of the illness, while the percentage of positive blood cultures was low at the beginning. The latter showed 92.9% and reached their maximal height first on the fifth day of the illness.

The filtering and arresting function of bone marrow against pathogenetic microbes in comparison with the circulating blood, seems to me to be something like a pool in comparison to a shoal in a river, i.e., the bone marrow is a pool and the streaming blood is a shoal. A man catches fish in a pool very much easier than in a shoal, even when he cannot see them. Out of 660 cases, 101 typhoid and 5 paratyphoid B cases could not be kept under examination by serial cultures following recovery, owing to death or discharge from our hospital. 435 typhoid, 15 paratyphoid A and 104 paratyphoid B cases were examined from beginning to

I

62 Years, ♀

Marrow Bacilli Carrier ; Vaccinated against Typhoid, May, 30, 1942

38	45	51	58	65	72	79	86	93
IV	VII	VIII	IX	X	XI	XII	XIII	XIV
$+_3$	$+_2$	−	$+_1$	$+_2$	$+_2$	$+_2$	$+_1$	$+_1$
−	−	−	$+_2$	−	−	−	−	−
−	−	−	−	+	+	+	+	+
+	+	+	−	−	−	−	+	−
−	−	−	−	−	−	−	−	−
T 500	T 500	T 500	T 500	T 800	T 800	T 800	T 800	T 500
A −	A −	A −	A −	A −	A −	A −	A −	A −
B −	B −	B −	B −	B −	B −	B −	B −	B −
−	−	−	+	−	−	−	−	−
−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−
+	−	−	−	−	−	−	−	−
38.0	37.4	36.6	38.8	38.3	38.0	36.8	36.7	36.8
		Fever free	56th day of the illness Revival				Fever free	Fever free

end. From the results of the examinations we understand how the development of bacilli in the bone marrow and blood vessels occurs.

Table III reveals the development of bacilli in the bone marrow and blood vessels. The bacilli were isolated from typhoid bone marrow in the first week in 91.9%, the second week in 81%, the third week in 64%, the fourth week in 40.3%, the fifth week in 24.1%, the sixth week in 13.4%, the seventh week in 7%, the eighth week in 4.4%, the ninth week in 2.8%, the tenth week in 2.3%, the eleventh week in 1.2%, the twelfth week and the thirteenth week in 0.9%, and the fourteenth week in 0.7%.

In paratyphoid the bacilli disappeared from the bone marrow much sooner than in typhoid. Paratyphoid A bacilli were present in bone marrow in the first week in 100%, the second week in 88.9%, the third week in 71.4%, the fourth week in 6.7%, and paratyphoid B bacilli, in the first week in 93.3%, the second week in 65.9%, the third week in 24.8%, the fourth week in 6.6%, the fifth week in 2.9% and the seventh week in 1%.

Figs. 1–3 show the reciprocal relation between fever and the positive findings of the bone marrow culture.

TABLE II

The Positive Results of Bone Marrow Culture in the First 7 Days from the Onset of the Illness

Day of the illness		1	2		3		4		5		6		7	
Results of cultures			Positiv											
		Cases %	Cases	%	Cases	%	Cases	%	Cases	%	Cases	%	Cases	%
No. of cases			1		9		12		14		22		53	
Typhoid	Bone marrow		1	100	9	100	10	83.3	13	92.9	20	90.9	49	92.5
	Blood		0		7	78.8	9	75.0	13	92.9	17	77.3	39	73.6
	Stool		0		1	11.1	4	33.3	3	21.4	7	31.8	19	35.9
	Urine		0		1	11.1	0		1	7.2	1	4.6	3	5.7
	Pharyngeal mucus		0		0		0		0		0		1	1.9
No. of cases							2		5		10		11	
Paratyphoid A ahd B	Bone marrow						2	100	4	80	10	100	11	100
	Blood						2	100	2	40	7	70	4	36.4
	Stool						1	50	2	40	4	40	5	45.5
	Urine						0		0		1	10	1	9.1
	Pharyngeal mucus						0		0		0		0	

2. *Presence of the bacilli in the bone marrow after the termination of fever*

Now there is the question: How long are the bacilli present in bone marrow after the afebrile period? In my serial cultures following recovery the bone marrow culture was continued after the afebrile period in typhiod in 261 cases, in paratyphoid A in 12 cases, and in paratyphoid B in 72 cases. From observation of cultures in the afebrile period there were 322 of 1727 cultures in typhoid, 17 of 46 cultures in paratyphoid A, and 83 of 262 cultures in paratyphoid B, in which the examinations of cultures were continued till the afebrile period (Table IV). The positive findings of these cultures in the afebrile period were 41 out of 536 typhoid cases (17%), 4 out of 15 paratyphoid A cases (33.3%), and 3 out of 109 paratyphoid B cases (4.2%), and from cultures in typhoid 19.6% (63 cultures), in paratyphoid A 35.3% (6 cultures), and in paratyphoid B 3.6% (3 cultures).

On the other hand the blood cultures in the afebrile period showed positive results in typhoid only in 3.3% of all cases and 3.1% of all cultures and in paratyphoid A and B no positive results.

In the afebrile period the positive sternal cultures showed their maximum in the third and fourth weeks of the illness. Observed during the weeks after the termination of fever (Table V), the bacilli in the bone

TABLE III

The Development of Pathogenetic Microbes in Bone Marrow and Blood Vessels in Every Week of the Typhoid and Paratyphoid Cases

	Week of the illness		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
Typhoid	Number of cases		111	357	432	435	441	433	431	432	432	435	432	433	434	435	435
	In bone marrow	No. of cases	102	289	278	175	106	58	30	19	12	10	5	4	4	3	0
		%	91.9	81.0	64.4	40.2	24.1	13.4	7.0	4.4	2.8	2.3	1.2	0.9	0.9	0.7	
	In blood	No. of cases	85	170	127	64	34	14	10	4	5	1	2	1	0	0	0
		%	76.6	47.6	29.4	14.7	7.7	3.3	2.3	0.9	1.2	0.2	0.5	0.2			
Paratyphoid A	Number of cases		1	9	14	15	15	15	15								
	In bone marrow	No. of cases	1	8	10	5	3	1	0								
		%	100	88.9	71.4	33.3	20.0	6.7									
	In blood	No. of cases	0	4	3	1	2	0									
		%		44.4	21.4	6.7	13.3										
Paratyphoid B	Number of cases		27	82	101	106	104	102	104	104							
	In bone marrow	No. of cases	26	54	25	7	3	0	1	0							
		%	96.3	65.9	24.8	6.6	2.9	0	1.0								
	In Blood	No. of cases	15	16	5	0	2	0									
		%	55.6	19.5	5.0	0	1.9	0									

marrow were positive in the first week in 41 typhoid cases, 4 paratyphoid A cases, and 3 paratyphoid B cases, and in the second week 14 typhoid cases, 2 paratyphoid A cases, but in paratyphoid B cases they were no longer positive.

3. *Bone marrow carrier*

After the termination of fever the bacilli gradually disappeared from the bone marrow,[1] but the longest duration of the bone marrow carrier state was six weeks, and in the seventh week the bacilli were no longer isolated.

The positive findings of the bacilli after the afebrile period usually depend upon the age of the patients. The older the age of the patients, the higher was the probability of positive culture, as Table VI shows. It is necessary to pay particular attention to the fact that in the bone marrow of those who had been discharged from hospitals under the negative results of stool and urine cultures, sometimes bacilli could be cultured. Such persons are called bacilli carriers, in whose stool and urine the bacilli are

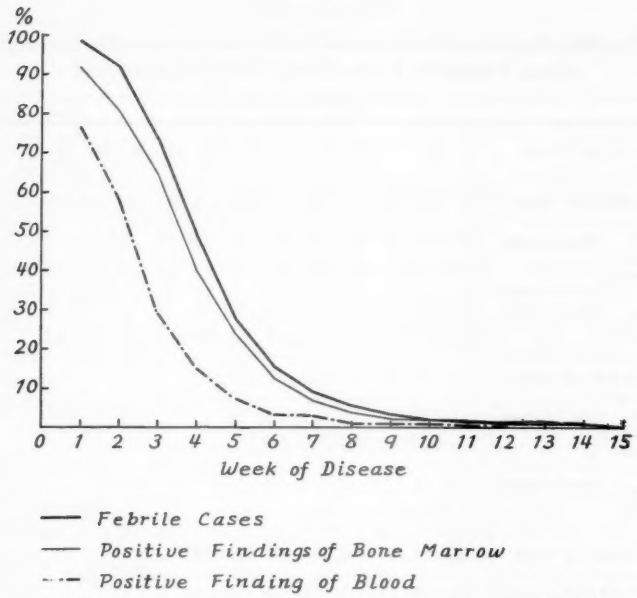

Fig. 1. The relation between fever and the development of the bacilli in bone marrow and blood in 536 typhoid cases.

found from the third week after termination of the fever (afebrile period). In the same way, if the nomenclature " bone marrow bacilli carrier " is admitted, 10 so-called typhoid- and 1 para typohid A bone marrow carriers were discovered by our examination. In this meaning Table VII shows the frequency of the positive cultures in the afebrile period and also the number and percentage of the bacilli carriers at the time of discharge from our hospital. In the afebrile period the bacilli were cultured from bone marrow in typhoid 7.7% ,in paratyphoid A 26.6%, and in paratyphoid B 2.8%. At discharge the bone marrow carriers were found in typhoid 1.9%, and in paratyphoid A 6.7%, but the bacilli were cultured out from the circulating blood only in 1.5% in the afebrile period and in 0.6% at discharge.

The positive findings of the bone marrow cultures in the afebrile period seemed to be influenced somewhat by the seasons: in Sendai, in northern Japan, after the afebrile period the bacilli tend to be cultured from bone marrow somewhat more easily in the cool season from September to April than in other seasons. It is probable that they are directly affected in the bone marrow by vital functions of the body.

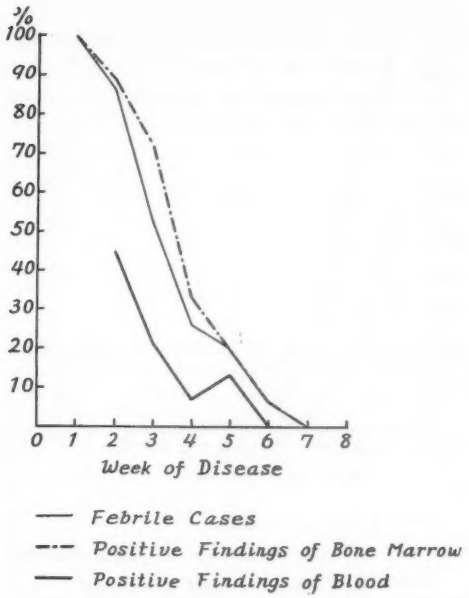

Fig. 2. The relation between fever and the development of the bacilli in bone marrow and blood in 15 paratyphoid A cases.

Due to unavoidable reasons 7 of these 11 bone marrow carriers could not be examined after leaving our hospital, so only 4 were examined repeatedly until cultural findings of their bone marrow turned out to be negative. They are as follows: Case I. C.F., farmer, aged 48, moderately severe typhoid, was admitted to our hospital in the fourth week of the disease. Prior to admission he had been treated for acute peritonitis. Cultural examinations of typhoid bacilli in bone marrow after admission always showed positive findings, but the bacilli were culturally positive in the blood only in the second and fourth weeks after the termination of fever, and only in a small number of colonies. It seemed as if they had been at first arrested in the bone marrow, and later entered the blood stream. In the third week after the termination of fever he left the hospital as a carrier of the bacilli in his bone marrow, and early in the seventh week they all disappeared from the marrow. Case II. S.K., housewife, aged 29, moderately severe typhoid. The bacilli were cultured only from bone marrow until the third week after the termination of fever (eleventh week of the illness), so she was discharged as a bone marrow bacilli carrier. In the seventh week after the termination of fever (fifteenth week of the illness) the bacilli were free from her bone marrow. Case III. I.R.,

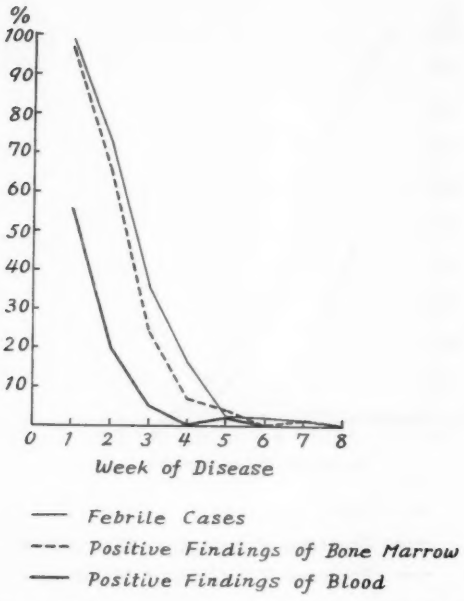

Fig. 3. The relation between fever and the development of the bacilli in bone marrow and blood in 109 paratyphoid B cases.

man, aged 49, severe typhoid. As the bacilli were positive in his bone marrow in the third week after the termination of fever (twelfth week of the illness) he was discharged as a bone marrow bacilli carrier. The bacilli were negative in his bone marrow early in the fourth week of the afebrile period. Case IV. A.K., man, aged 40, severe typhoid. He was discharged as a bone marrow bacilli carrier in the third week of the afebrile period, and in the eleventh week (seventeenth week after the start of the illness) his bone marrow was culturally negative.

As mentioned above the longest duration of the existence of the bone marrow bacilli is six week after the termination of fever.

4. *Clinical significance of bone marrow cultures in making diagnosis of typhoid and paratyphoid*

It is clear that the bone marrow culture is very important in making an early diagnosis of typhoid and paratyphoid, for on admission there were 95 of 536 typhoid cases (17.7%), 4 of 15 paratyphoid A cases (26.7%), and 25 of 109 paratyphoid B cases (22.9%), in which the bacilli were cultured only from bone marrow. In the duration of the illness there were 48 typhoid (9%), 2 paratyphoid A (13.2%) and 18 paratyphoid B

TABLE IV

The Positive Findings of Bone Marrow, Blood, Stool, Urine and Pharygeal Mucus in the Afebrile Period

(a) **The Positive Cases in the Afebrile Period** (from Cases)

Species	Cases in which serial cultures were made in the afebrile period	Bone marrow		Blood		Stool		Urine		Pharyngeal mucus	
		Cases	%	Cases	%	Cases	%	Cases	%	Cases	%
T.A.	241	41	17.0	8	3.3	71	29.5	25	10.4	6	2.5
P.A.	12	4	33.3	0		4	33.3	1	8.3	1	8.3
P.B.	72	3	4.2	0		22	30.6	9	12.5	3	4.2

(b) **The Positive Examinations in the Afebrile Period** (from Cultures)

Species	No. of cultures which were made in the afebrile period	Bone marrow		Blood		Stool		Urine		Pharyngeal mucus	
		Cultuees	%	Cultures	%	Culture	%	Cultures	%	Cultures	%
T.A.	322	63	19.6	10	3.1	84	26.1	34	10.6	9	2.8
P.A.	17	6	35.3	0		3	17.7	2	11.8	1	5.9
P.B.	83	3	3.6	0		25	30.1	9	10.8	3	3.6

TABLE V

The Positive Findings of the Bone Marrow Culture after the Termination of Fever (Cases)

Week after the termination of fever	I	II	III	IV	V	VI	VII
T. A.	41	14	9	1	1	1	0
P. A.	4	2	1	0			
P. B.	3	0					

(16.5%) cases, in which the bacilli were cultured only from the bone marrow.

There were not a few cases, in which on admission Widal's reaction remained negative or not high enough to make a difference between a diagnosis of typhoid and paratyphid. Among my cases there were 65 typhoid (12%), in which the titer of Widal's reaction of typhoid was equal to or lower than paratyphoid, 12 paratyphoid A (80%), in which the titer of paratyphoid A was equal to or lower than paratyphoid B or typhoid, and 39 paratyphoid B (35.7%), in which the titer of paratyphoid B was equal to or lower than paratyphoid A or typhoid.

It is particularly note-worthy that there are many paratyphoid A cases, in which the titer of Widal's reaction for pratyphoid A was on ad-

TABLE VI

Relation between the Age of the Patients Whose Bone Marrow Cultures were Positive in the Afebrile Period and the Occurence of the Bone Marrow Bacilli Carrier

Species	Age groups	0～10	11～20	21～30	31～40	41～50	51～and over
T.A.	Cases, in which bone marrow cultures were made	110	190	98	67	40	31
	Positive cases in the afebrile period	5	13	5	6	6	6
	Bone marrow bacilli carrier	0	0	1	2	4	3
P.A.	Cases, in which bone marrow cultures were made	0	4	7	2	1	1
	Positive cases in the afebrile period	0	0	4	0	0	0
	Bone marrow bacilli carrier	0	0	1	0	0	0
P.B.	Cases, in which bone marrow cultures were made	42	46	5	14	2	0
	Positive cases in the afebrile period	3	0	0	0	0	0
	Bone marrow bacilli carrier	0	0	0	0	0	0

TABLE VII

The Frequency of the Positive Bone Marrow Cultures in the Afebrile Period and the Frequency of the Bone Marrow Carrier

Species	Cases in which serial cultures were made	Cases in which bone marrow cultures were positive in the afebrile period				Bone marrow carriers			
		Bone marrow		Blood		Bone marrow		Blood	
		Cases	%	Cases	%	Cases	%	Cases	%
T.A.	536	41	7.7	8	1.5	10	1.9	3	0.6
P.A.	15	4	26.7	0		1	6.7		
P.B.	109	3	2.8	0		0			

mission not higher than that for typhoid or paratyphoid B. Among my cases 9 paratyphoid A cases (60%) and 37 paratyphoid B cases (33.9%) were sent to our hospital as typhoid cases. On the contrary, 24 typhoid cases (4.5%) were sent as paratyphoid.

Furthermore I had 54 cases, which were admitted as typhoid or paratyphoid, but by means of the bone marrow culture and from clinical features they were diagnosed correctly without difficulty. They were chiefly typhus, Japanese B encephalitis, meningitis tuberculosa, common

cold or influenza, sepsis, pulmonary tuberculosis and others.

From one case of sepsis, admitted to the hospital as a typhoid patient, streptococci were isolated, and from two other cases staphylococci were isolated by means of the bone marrow culture. In microscopic examination of one malaria case and two leishmaniosis cases I found pathogenetic parasites in the bone marrow.

Bock[2] has maintained that in septic diseases the sternal culture showed better results than the venous blood, but that the former was inferior to the arterial blood culture. My cultural findings of 54 typhoid and paratyphoid cases showed on admission that the percentage of the bone marrow (sternal) culture was 72%, the arterial blood culture 53% and the venous blood culture 29%.

That is to say, in typhoid and paratyphoid the bone marrow culture is the best for isolation of the pathogenetic microbes in almost every period of the disease.

I usually try the bone marrow puncture for injection of various kinds of chemicals and blood transfusion, when venous and arterial punctures are almost impossible. Also in cases when the patients are too fat, too weakened or fall into collapse, when after repeated punctures the veins have become too hard, and in the case of children and nervous women, whose veins are too fine.

Concerning places for the bone marrow puncture I recommend, in place of the brest bone, the tibia in children, and in adults, particularly nervous women, the spine or the crest of the ilium.

Conclusions

As became clear to me in my examinations, the bone marrow culture is very valuable in making diagnosis in cases of typhoid and paratyphoid, when it is required to make an early diagnosis soon after the onset of the illness.

It is note-worthy also, when the Widal's reaction is persistently negative or group agglutinations are present, that Widal's reaction gives no aid in differentiating typhoid from paratyphoid. Furthermore when patients have been vaccinated or have once suffered from typhoid or paratyphoid (reinfection), this reaction then has no practical value in differentiating between typhoid or paratyphoid and other acute febrile diseases.

The bone marrow culture was performed easily and safely in all cases without exception and the results of the bone marrow culture were always excellent in comparison with those of the blood culture and others.

In my 660 typhoid and paratyphoid cases 2035 bone marrow cultures were made and the development of the patogenetic microbes in bone

marrow and the relation between the cultural findings of bone marrow and clinical symptoms were observed.

The bone marrow culture is the best for isolation of the pathogenetic microbes in typhoid and paratyphoid in comparison with arterial and venous blood, stool, urine and pharyngeal mucus.

The bone marrow puncture is very valuable not only in hematological and bacteriological diagnosis but also in the purpose of treatment, when venous and arterial punctures are almost impossible.

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Selective Stimulation of Retinal Receptors with Electric Currents of Various Forms

By

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Introduction

It has long been known that a subjective sensation of light, electrical phosphene, can be aroused by an electric stimulus passing through the eye[1-3].

Much work has been done by a number of investigators[4-9] on the effect of a direct current upon the electrical sensitivity of the retina. Some authors[10-13] determined also the threshold strengths for alternating currents of varying frequencies passing through the eye as determined with flickering phosphenes as an index.

Motokawa and his associates[14-17] have recently provided evidence that an alternating current has a selective excitatory action upon the retinal receptors, according to its frequency in human as well as animal experiments.

In the present investigation it was attempted, therefore, to study whether direct currents of various forms have such a selective excitatory action as that of an alternating current. Electric stimuli used were condenser discharges (C.D.), exponentially increasing currents (E.I.C.) of varying time constants, and rectangular pulses (R.P.) of varying durations.

Experimental

Method

Motokawa's method consists in measuring the electrical excitability of the eye after exposure to light, taking the least perceptible phosphenes as an index.

In our experiments an electric current of a given form was used instead of a light stimulus for conditioning, and its effect was tested by means of a constant current pulse of 0.1 second at different times after

the conditioning stimulus.

The details of the apparatus and the procedure to determine the electrical excitability of the eye are described in Motokawa's papers.[18-20]

Electric stimuli were applied to the eye through a pair of silver electrodes of 2×1.5 cm^2 in size, the cathode placed on the forehead slightly above the eyebrow and the anode on the homolateral temple of the subject.

The contact of the electrodes with skin was secured by the use of warmed electrode paste nearly saturated with NaCl.

All experiments were performed in a dark room after a preliminary dark adaptation of about 20 minutes. The eye was stimulated with the conditioning electric stimulus and at varying intervals thereafter the eye was stimulated with a single constant curent pulse to determine its electrical excitability, E.

The procedure for determining a threshold is as follows:

Starting with a voltage of 2–3 volts which is sufficient to cause a distinct phosphene, the stimulating voltage was reduced step by step. When the voltage is so much reduced that the subject feels it difficult to distinguish an electrical phosphene from the back ground of intrinsic light of the eye, the subject demands a comparing procedure. The procedure consists in delivering two electric stimuli to be compared, that is, the stimulus in qestion and the control stimulus far below the threshold. The subject is requested to answer the question which the stronger stimulus is, and informed every time whether his answer is right or wrong at the time. When an answer given by the subject is right, the same comparing procedure is repeated with stimulating voltages reduced further until the discrimination fails. If the answer given by the subject is wrong, the test with the same stimulating voltage is repeated 3 times. It is decided that he can no longer distinguish the two stimuli if he fails twice.

The threshold so determined is one of the thresholds, and not always the lowest one. Try to stimulate the eye again with a voltage reduced about 5% after a threshold has been reached, so the subject will sometimes report that a phosphene is perceptible. He will succeed in discriminating the stimulus from the control one. In such cases the same comparing procedure should be repeated with further reduced voltages until the second threshold is reached. In this manner, two or more thresholds can be determined. Since the lowest one is a true threshold, we should endeavor to get the lowest threshold under a given experimental condition. For this purpose it is necessary to make some tests with further reduced voltages after a threshold is reached, in order to be sure that it is a true threshold.

The strength of the conditioning electric stimulus was made about

10% below threshold, because it was found in a preliminary experiment that the effect expressed in terms of ζ was greatest when the intensity of the conditioning stimulus was near the threshold. If a stimulus above threshold is used for conditioning it would be difficult to distinguish the phosphene caused by the conditioning stimulus from that caused by the test stimulus. This is the second reason for adopting a subthreshold conditioning stimulus.

The effect of a conditioning electric stimulus consists of supernormal electrical excitability, whose time course is very similar to that caused by a photic conditioning stimulus. Therefore the effect can be expressed in terms of ζ in the same manner as in Motokawa's experiments. ζ is defined by the following formula: $\zeta = 100\ (E\text{-}E_0)/E_0$, where E and E_0 mean electrical excitabilities determined with and without a conditioning stimulus.

The circuits for producing a C.D., an E. I. C. and a R. P. are illustrated in Fig. 1 A, B and C respectively. In these diagrams the circuits comprising S_1 and S_2 are for production of test stimuli. The voltage of a test stimulus is controlled by a variable resistance contained in the circuits. The switches S_1 and S_2 are opened at an interval of 0.1 second by means of a rheotome to produce a rectangular pulse of 0.1 second in duration.

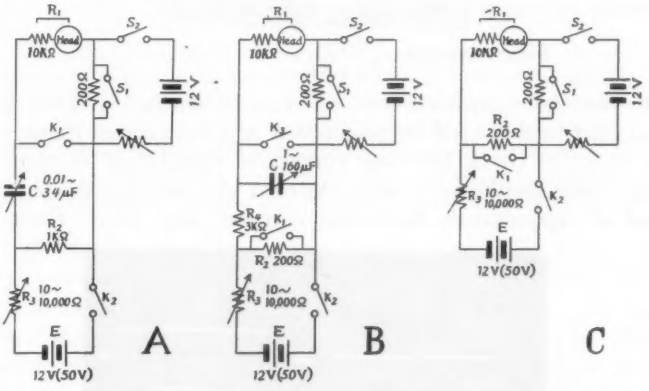

Fig. 1. A, circuit used for production of C. D. B, circuit used for production of E. I. C. C, circuit used for production of R. P.

Circuit containing S_1, S_2 and 12 volts of each diagram is the one for producing a test single pulse. R_1: sum of the resistance of the head and 10 kiloohms.

The circuit for production of a C.D. is shown in Fig. 1 A. By a procedure of closing the switches K_1 and K_2, and opening the switches K_1 and K_2 in the order, the condenser C is discharged through the circuit. If the resistance of

the circuit containing the head of the subject be R_1, the change of voltage at this resistance will be expressed by:

$$V_H = \frac{R_1 V}{R_1+R_2} \exp\left[-\frac{t}{C(R_1+R_2)}\right], \text{ where}$$

$$V = \frac{ER_2}{R_2+R_3}$$

Since R_2 is negligibly small against R_1, the time constant of the condenser discharge is approximately CR_1.

The circuit for production of an E.I.C. is shown in Fig. 1 B. By a procedure of closing the switches K_1 and K_2, and opening the switch K_1 in the order, an E.I.C. is applied to the head. The interval between opening the switch K_1 and closing the switch K_3 is fixed at 0.5 seconds.

The change of voltage in the head circuit is expressed by:

$$V_H = \frac{R_1 V}{R_1+R_4+Z}\left[1-\exp-\frac{t}{\frac{CR_1(R_4+Z)}{R_1+R_4+Z}}\right], \text{ where}$$

$$V = \frac{ER_2}{R_2+R_4} \quad \text{and} \quad Z = \frac{R_2R_3}{R_2+R_3}$$

$$\text{Time constant} = \frac{CR_1(R_4+Z)}{R_1+R_4+Z}$$

Since Z is sufficiently small compared with R_4, the time constant of the exponentially increasing current will be expressed by:

$$\text{Time constant} = \frac{CR_1R_4}{R_1+R_4}$$

The circuit for production of a R.P. is shown in Fig. 1 C. This circuit is similar to that used for production of a test current pulse.

In order to check the time courses of stimulating voltages experimentally, some oscillograms were recorded of condenser discharges (Fig. 2A) and of exponentially increasing currents (Fig. 2B). These records

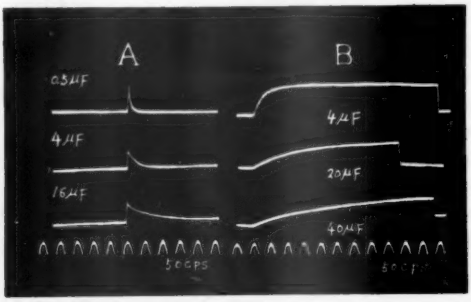

Fig. 2. A and B represent electromagnetic oscillograms of C. D. and E.I.C. under the same conditions as in the experiment. Number on each record indicates the capacity of condenser used in μF.

show that the time courses of both kinds of stimuli are prolonged with increases in capacity, as is predicted by theoretical formulas mentioned above. However, the records shown in Fig. 2 were obtained by means of an electromagnetic oscillograph, which is too slow an instrument for recording rapid changes, especially in the initial phase of the voltage changes. So some records were obtained by the use of a cathode-ray oscillograph (Fig. 3). In diagrams A, E and G of this figure a physical

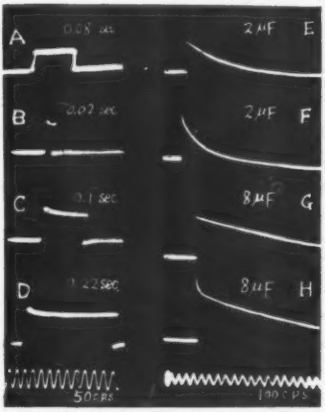

Fig. 3. A, cathode-ray oscillogram of R. P. taken with a physical resistance of 10 kiloohms instead of the head. B, C and D, cathode-ray oscillograms of R. P. taken under the same conditions as in the experiment. Number on each record indicates the duration of R.P. in sec. E and G, cathode-ray oscillograms of C.D. taken with a physical resistance of 10 kiloohmes instead of the head. F and H, records of C.D. taken under the same conditions as in the experiment. Number on each record indicates the capacity in μF.

resistance 10 kiloohms were used instead of the head of the subject so that they might serve as controls to the other diagrams recorded under the same experimental conditions as in the following experiments. As can be seen in these diagrams some distortion takes place due to skin polarization, but this situation hardly seems to change the general feature of the problem.

Results

1. Experiments with condenser discharge.

In the first series of experiments the effect of subthreshold conditioning C.D. upon the electrical excitability of the eye was studied. In this experiment the eye was stimulated by the subthreshold conditioning C.D. of varying time constants ranging from 0.0002 to 0.68 seconds, and the changes in electrical excitability of the eye were measured at varying

intervals from the onset of the conditioning stimulus. The result is illustrated in Fig. 4, in which ζ values are plotted against intervals between the onset of the conditioning stimulus and the test stimulus. The time constant of each condenser discharge is given in seconds.

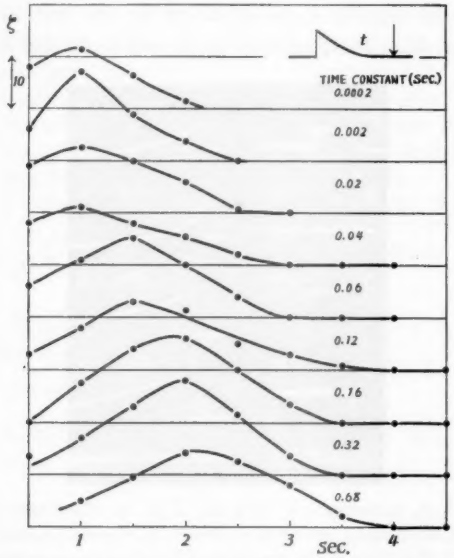

Fig. 4. Changes in electrical excitability of human eye following subthreshold conditioning C. D. Ordinates: percentage increases of electrical excitability above resting level. Abscissas: time in sec. from the onset of the conditioning electric stimulus to the test stimulus. Number on each curve indicates the time constant of C.D. in sec.

As can be seen in Fig. 4, the dependence on the time constant of the conditioning C. D. is such that the crest times of ζ-time curves become longer as the time constant of the conditioning stimulus is increased. Such displacements of the crest were observed by Motokawa[18], when he used colored lights of varying wavelengths as conditioning stimuli; the crest time became longer as the wavelength of the conditioning light became shorter.

The present experiment shows, therefore, that the effect of the subthreshold conditioning C.D. is similar to that of colored light.

From comparison of the crest times in both cases we may conclude that C.D. of shorter—from 0.0002 to 0.04 seconds—, intermediate—from 0.06 to 0.12 seconds—and longer—from 0.16 to 0.68 seconds—time constants are equivalent to red, yellow and green lights respectively. In

other words, it is likely that in stimulation with C.D. the chief retinal receptor involved would be the red, yellow and green receptors respectively as the time constant of the C.D used is increased.

The term "receptor" has been used to represent a functional unit of retinal organization from the receptor cells to the optic fibers.

2. Experiments with exponentially increasing currents.

In the second series of experiments the effect of subthreshold conditioning E.I.C. upon the electrical excitability of the eye was studied. In this series, the interval between the "make" and the "break" of the stimulating current was fixed at 0.5 seconds.

The result is illustrated in Fig. 5, in which ζ values are plotted as ordinates against time after the onset of the conditioning stimulus as abscissas. As can be seen in this figure, the crest of ζ-time curve is displaced from 2 seconds toward 3 seconds as the time constant of the conditionig stimulus is increased from 0.0026 toward 0.08 seconds. With larger time constants, there appear two elevations, one having a crest at 3 seconds and the other at 4.5 seconds.

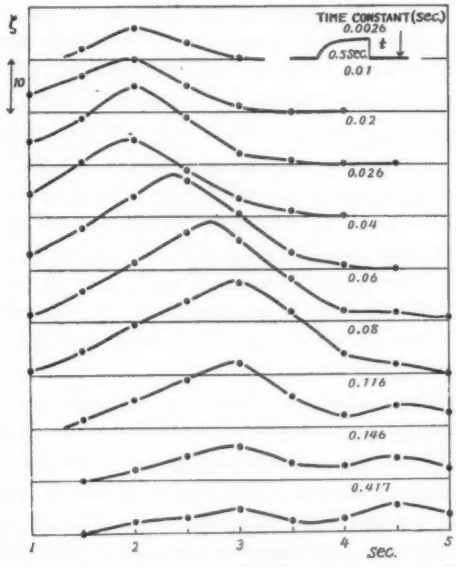

Fig. 5. Changes in electrical excitability of human eye following subthreshold conditioning E.I.C. Ordinates: percentage increases of electrical excitability above resting level. Abscissas: time in sec. from the onset of the conditioning electric stimulus to the test stimulus. Number on each curve indicates the time constant of E.I.C. in sec.

In the experiments by Motokawa[16)21)] green, blue-green and blue conditioning lights produced ζ-time curves having crests at 2, 2.5, and 3 seconds respectively. Therefore, the E.I.C. of time constants ranging from 0.0026 to 0.026 seconds may be equivalent to green light, those with time constants from 0.04 to 0.06 seconds to blue-green light, and those with time constants from 0.08 to 0.417 seconds to blue light. It is to be noted that the E.I.C. can not have any effect equivalent to that of red light, however short the time constant may be.

We have observed above that a small elevation appeared at 4.5 seconds in the ζ-time curves following subthreshold E.I.C. of larger time constants. This elevation may be identified with the rod-process, because Motokawa and his associates[22-23)] showed that the ζ-time curve of rods had a crest at 4.5 seconds as measured from the onset of pre-illumination, and this was fully confirmed by Oikawa.[24)]

Since the rod-process is generally slower than the cone-processes, E.I.C. of larger time constants are effective to stimulate the scotopic receptor.

3. Experiments with rectangular pulses.

In the third series of experiments the effect of subthreshold conditioning R.P. upon the electrical excitability of the eye was studied. In this experiment the eye was stimulated by subthreshold conditioning R.P. of varying durations ranging from 0.0015 to 0.5 seconds, and then changes in electrical excitability were measured at varying intervals from the end of the subthreshold conditioning electric stimulus.

As can be seen in Fig. 6, the dependence on the time constant of R.P. is such that the crest time of ζ-time curves as measured from the end of the subthreshold conditioning electric stimulus becomes longer as the duration is increased. Compared with the effects of conditioning colored lights in Motokawa's experiments[18)], the R.P. of the durations ranging from 0.0015 to 0.0022 seconds may be equivalent to red light, those from 0.0037 to 0.03 seconds to yellow light and those from 0.213 to 0.5 seconds to green light.

In the next series of experiments, the effects of the strength of C.D., E.I.C. and R.P. were measured upon increases in the electrical excitability of the eye caused by the conditioning electric stimuli. The strength is expressed in multiples of threshold. The effect of the strength of the conditioning C.D. is illustrated in Fig. 7A. The interval between the onset of the conditioning electric stimulus and the test stimulus was made 1, 1.5 and 2 seconds as the time constants of the C.D. used were 0.02, 0.06 and 0.16 seconds respectively, because these intervals corresponded to the maxima of the respective ζ-time curves.

The strength of the conditioning E.I.C. was varied, and the data

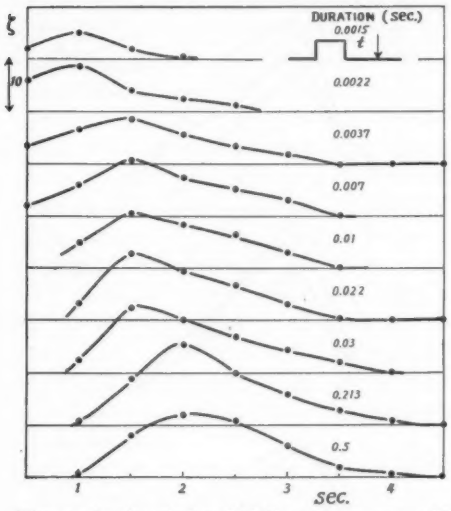

Fig. 6. Changes in electrical excitability of human eye following subthreshold conditioning R. P. Ordinates: percentage increases of electrical excitability above resting level. Abscissas: time in sec. from the end of the conditioning electric stimulus to the test stimulus. Number of each curve indicates the duration of R. P. in sec.

represented in Fig. 7 B was obtained. For the same reason, the interval between the onset of the conditioning electric stimulus and the test stimulus was fixed at 2, 2.5 and 3 seconds for the time constants of 0.02, 0.04 and 0.116 seconds respectively.

Similar data obtained with R.P. are shown in Fig. 7 C. The interval between the end of the conditioning electric stimulus and the test stimulus was fixed at 1, 1.5 and 2 seconds for the durations of 0.0022, 0.022 and 0.5 seconds respectively.

As can be seen in Fig. 7, irrespective of the kinds of wave-form of the conditioning electric stimuli, ζ values are greatest when conditioning stimuli of threshold strength are used. As the strength of the conditioning electric stimulus rises from subthreshold intensities, ζ values increase, attain a maximum at the threshold strength, and then decrease gradually with further increases in the intensity of conditioning stimulus. This is one of the reasons why conditioning stimuli a little below the threshold were used in the present experiments.

The question as to why too strong conditioning stimuli are ineffective is of great interest, but nothing can be said at present about this question.

In the next experiments, we studied the effects of the direction of

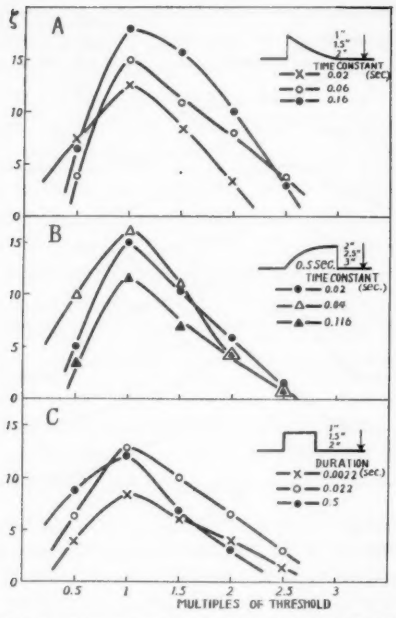

Fig. 7. Effects of the strength of C. D. (A), E.I.C. (B) and R.P. (C) upon increases in electrical excitability of the eye. Arrows in the insets indicate a test stimulus.

current flow upon the electrical excitability of the eye. We usually placed the cathode on the forehead for both conditioning and test stimuli. The direction of the conditioning stimulus was now made opposite to the direction of the test stimulus, and measurements were carried out under the same experimental conditions. The only effect of the change in the direction of the conditioning stimulus was a slight decrease of ζ value, without any alteration in the other relations.

Discussion

Motokawa[18] reported that the change in electrical excitability shows a time course characteristic of the wavelength of the light used for the conditioning photic stimulus. The crest times of ζ-time curves are 1, 1.5, 2 and 3 seconds for conditioning photic stimuli of red, yellow, green and blue lights respectively. This fact suggests that there are various kinds of receptors with varying time constants in the retina.

In general, it is a principle of electrophysiology that a mixture of excitable tissues with different velocity constants can selectively be stimulat-

ed using electric stimuli of varying forms and time constants. This has been demonstrated by the present investigation, for the displacements of the crest of the ζ-time curve mentioned above can be interpreted in this sense. It has been shown that C.D. were effective in shifting the crest from 1 second to 2 seconds, but failed to shift it further toward 3 seconds. This finding may be so interpreted that rapidly changing electric stimuli like C.D. act preferably upon the red, yellow and green receptors, but not upon the slowly responding blue receptor and rods. On the contrary, E.I.C. were found to be effective in stimulating the latter receptors, for the crests of the ζ-time curves were found at 3 and 4.5 seconds, when the time constants of the E.I.C. used were larger. The crest was found at 2 seconds for the shortest time constant of the E.I.C., and this was also the case with the experiment in which R.P. of longer duration were used for conditioning. These findings can well be understood when the situation is taken into consideration that the E.I.C. of the shortest time constant resembles the R.P. of 0.5 seconds in wave form.

As well-known, the threshold for a slowly increasing current is higher than for a rapidly increasing one. This change in threshold is called "accommodation."[25] Our finding suggests that the blue receptor and rods show slower accommodation than do the other cone-receptors, because E.I.C. of larger time constants have a selectively stimulating action upon the blue receptor and rods.

As has been revealed by Motokawa's method[18][22], red, yellow, green, blue and rod retinal processes are more rapid in the order. The C.D. are most effective for activating more rapid processes like the red, yellow and green processes, while the E.I.C. are favorable for exciting slower processes like the blue and rod processes. The R.P. are intermediate between them.

Thus, the retinal receptors are activated, to some extent, similarly by photic and electric stimuli. However, there are some differences between the modes of action of the photic and electric stimuli. For instance, when the photic stimulus is used for conditioning, the ζ value increases as the intensity of light rises. This is, however, not the case with the electric stimulus; as has been stated above, there is an optimal strength of the conditioning electric stimulus. This discrepancy may be due to the differences in seats attacked by adequate and non-adequate stimuli. The photic stimulus seems to act directly upon the receptor cells themselves, and the photochemical changes initiated by the photic stimulus will produce an electrical change.

As pointed out by some authors,[6][18][26] an electric stimulus seems to attack directly the retinal network without initiating any photochemical reaction. It may be supposed that a too strong electric stimulus would create some inhibitory change in the retinal network and depress the

excitability as tested by a following electric stimulus.

Summary

The eye was stimulated by subthreshold conditioning electric stimuli of various wave-forms, and then the changes in the electrical excitability of the eye were investigated by applying a single constant current pulse of 0.1 second in duration. Percentage increases in electrical excitability following the conditioning stimulus were denoted by ζ and plotted as ordinates against the time after the conditioning stimulus.

1) In experiments carried out with condenser discharge (C.D.) as conditioning stimulus, the ζ-time curve had a crest at 1 second for the time constants ranging from 0.0002 to 0.04 seconds, at 1.5 seconds for those from 0.06 to 0.12 seconds and at 2 seconds for those from 0.16 to 0.68 seconds.

2) When exponentially increasing currents (E.I.C.) were used as conditioning stimuli, the crest of the ζ-time curve was found at 2 seconds for the shortest time constant. The crest was shifted toward 3 seconds as the time constant was increased, and there appeared two crests at 3 and 4.5 seconds for the largest time constant.

3) Rectangular pulses from 0.0015 to 0.5 seconds in duration showed effects similar to those of C.D.

4) The effects stated in (1), (2) and (3) are closely similar to the effects of colored lights used instead of the conditioning electric stimuli. From comparison of the effects of both kinds of stimuli, it was concluded that the red and yellow receptors are selectively stimulated by C.D., while the blue and rod receptors are excited by E.I.C. Rectangular pulses seemed to have an action intermediate between C.D. and E.I.C.

5) The effect of the intensity of the conditioning electric stimulus was studied upon ζ values, and it was found that the threshold strength was optimal. This is a striking contrast to the effect of photic stimuli which always increases with increasing intensities.

I wish to express my thanks to Prof. K. Motokawa for suggesting this investigation as well as for constant guidance in the course of the work.

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